# THE BOOK WAS DRENCHED

UNIVERSAL
LIBRARY

# MONOGRAPHS ON BIOCHEMISTRY

EDITED BY
R. H. A. PLIMMER, D.Sc.
AND
SIR F. G. HOPKINS, M.A., M.B., D.Sc., F.R.S.

## GENERAL PREFACE.

THE subject of Physiological Chemistry, or Biochemistry, is enlarging its borders to such an extent at the present time, that no single textbook upon the subject, without being cumbrous, can adequately deal with it as a whole, so as to give both a general and a detailed account of its present position. It is, moreover, difficult in the case of the larger textbooks to keep abreast of so rapidly growing a science by means of new editions, and such volumes are therefore issued when much of their contents has become obsolete.

For this reason an attempt is being made to place this branch of science in a more accessible position by issuing a series of monographs upon the various chapters of the subject, each independent of and yet dependent upon the others, so that from time to time, as new material and the demand therefor necessitate, a new edition of each monograph can be issued without re-issuing the whole series. In this way, both the expenses of publication and the expense to the purchaser will be diminished, and by a moderate outlay it will be possible to obtain a full account of any particular subject as nearly current as possible.

The editors of these monographs have kept two objects in view: firstly, that each author should be himself working at the subject with which he deals; and, secondly, that a *Bibliography*, as complete as possible, should be included, in order to avoid cross references, which are apt to be wrongly cited, and in order that each monograph may yield full and independent information of the work which has been done upon the subject.

R. H. A. P.
F. G. H.

# MONOGRAPHS ON BIOCHEMISTRY

ROYAL 8VO

EDITED BY

R. H. A. PLIMMER, D.Sc.

AND

SIR F. G. HOPKINS, D.Sc., F.R.S.

THE CARBOHYDRATES. By E. F. ARMSTRONG, D.Sc., F.R.S., and K. F. ARMSTRONG, M.A., B.Sc. 15s. net.

THE GLYCOSIDES. By E. F. ARMSTRONG, D.Sc., F.R.S., and K. F. ARMSTRONG, M.A., B.Sc. 12s. 6d. net.

THE NATURE OF ENZYME ACTION. By Sir W. M. BAYLISS, D.Sc., F.R.S. 9s. net.

THE PHYSIOLOGY OF PROTEIN METABOLISM. By E. P. CATHCART, M.D., D.Sc., F.R.S. 12s. 6d. net.

ENZYMES. By J. B. S. HALDANE, M.A. With 35 Diagrams. 14s. net.

ALCOHOLIC FERMENTATION. By ARTHUR HARDEN, D.Sc., F.R.S. 15s. net.

CREATINE AND CREATININE. By ANDREW HUNTER, M.B., F.R.S. 14s. net.

NUCLEIC ACIDS: Their Chemical Properties and Physiological Conduct. By WALTER JONES, Ph.D. With 3 Illustrations. 9s. net.

THE PROTAMINES AND HISTONES. By the late ALBRECHT KOSSEL. Translated from the Original German Manuscript by WILLIAM VEALE THORPE, M.A., Ph.D. 9s. net.

HEXOSAMINES AND MUCOPROTEINS. By P. A. LEVENE. 10s. 6d. net.

LECITHIN AND ALLIED SUBSTANCES: The Lipins. By HUGH MACLEAN, M.D., D.Sc., and IDA SMEDLEY MACLEAN, D.Sc., F.I.C. 10s. 6d. net.

THE VEGETABLE PROTEINS. By THOMAS B. OSBORNE, Ph.D., Sc.D. With 13 Diagrams. 9s. net.

BACTERIAL METABOLISM. By MARJORY STEPHENSON, M.A. With 34 Diagrams. 18s. net.

THE CHEMISTRY OF UREA: The Theory of its Constitution, and the Origin and Mode of its Formation in Living Organisms. By EMIL A. WERNER, Sc.D., F.I.C. 14s. net.

# THE CARBOHYDRATES

BY

E. F. ARMSTRONG, D.Sc., Ph.D., LL.D., F.R.S.

AND

K. F. ARMSTRONG, M.A., B.Sc.

LONGMANS, GREEN AND CO.

LONDON • NEW YORK • TORONTO

LONGMANS, GREEN AND CO. LTD
39 PATERNOSTER ROW, LONDON, E.C. 4
6 OLD COURT HOUSE STREET, CALCUTTA
53 NICOL ROAD, BOMBAY
36A MOUNT ROAD, MADRAS

LONGMANS, GREEN AND CO
114 FIFTH AVENUE, NEW YORK
221 EAST 20TH STREET, CHICAGO
88 TREMONT STREET, BOSTON

LONGMANS, GREEN AND CO.
480 UNIVERSITY AVENUE, TORONTO

## *BIBLIOGRAPHICAL NOTE*

| | | |
|---|---|---|
| *First Edition in Combined Volume with "The Glucosides"* | *March* | *1910* |
| *Second Edition* | *September* | *1912* |
| *Third Edition* | *August* | *1919* |
| *Fourth Edition* | *July* | *1924* |
| *Fifth Edition in Separate Volumes* | *November* | *1934* |

(*"The Glucosides" published separately November 1931*)

***Printed in Great Britain***

## PREFACE TO FIFTH EDITION.

THE present work completes the revision of "The Simple Carbohydrates and the Glucosides" begun by the publication of "The Glycosides" in 1931. The work has been out of print for some time, but we have waited until some degree of certainty has been reached in controversial matters of structure before bringing the book up-to-date.

Since the last edition was published, a vast amount of new knowledge in sugar chemistry has been accumulated; the work has been completely rewritten. It is primarily our object to appeal to those interested in sugar chemistry from the biochemical aspect, and we have therefore confined our attentions to the natural sugars and their derivatives and restricted as much as possible the discussion of intricate structural problems.

Thanks to the work of Haworth and his school at Birmingham, the formulæ of the two ring forms of glucose have at last been definitely established. From this certain basis has been developed an exact knowledge of the structure of disaccharides, the oligosaccharides and even the high molecular polysaccharides. It is not too much perhaps to say that the main essentials of the different types of structural combination adopted by sugar derivatives in nature have been established, and that new work may be predicted to fit into the present scheme but will not alter it. Much remains to be done, especially in the polysaccharides, which have recently come into prominence for their importance in immunology.

We have in the main restricted our treatment to the

sugars as static structures, on which a discussion of sugar transformation must be based, and have purposely avoided a discussion of sugar metabolism, muscle contraction and fermentation, for one reason that the subject is still very controversial and being constantly upset, and for another that the different points of view have been represented in recent monographs by the workers in these subjects themselves.

The sugars have attracted workers of every nationality, and so many schools have contributed to the advancement of the knowledge concerning them that it is impossible to mention individual names in this preface. Emil Fischer would have been well pleased to see that there had been no loss of interest in his favourite theme and satisfied that his own work had stood the test of time.

In order to facilitate future revision of our book, the authors would ask their colleagues to favour them with reprints of their publications.

69 Barkston Gardens,
London, S.W. 5,
*May* 1, 1934.

# CONTENTS.

# CHAPTER I.

## THE STRUCTURE OF GLUCOSE.

THE carbohydrate group includes the series of compounds of increasing molecular weight from the gas formaldehyde $CH_2O$, the simplest hydrate of carbon, to the highly polymerised starch and cellulose $(C_6H_{10}O_5)_n$, which possess very large molecules. The simplest true member of the sugar series which has both a carbonyl $>C=O$, and one or more alcoholic groups $-CH(OH)-$, is glycollic aldehyde $CH_2OH \cdot CHO$, a sweet tasting, crystalline substance readily soluble in water.

When hydrolysed by heating with acids, the complex carbohydrates or polysaccharides are resolved into the simple monosaccharides of which glucose is the typical representative.

These have the general formula $(CH_2O)_n$: in the natural sugars $n$ is either five—arabinose, xylose, ribose—or six—glucose, fructose, mannose, galactose. The disaccharides are derived from the monosaccharides by elimination of a molecule of water from two molecules of monosaccharide; they have thus the formula $C_{12}H_{22}O_{11}$ (when derived from two hexoses)—maltose, lactose, sucrose.

Similarly, the polysaccharides are formed by elimination of $n-1$ molecules of water from $n$ molecules of monosaccharide.

The members of the sugar group are usually distinguished by names having the suffix *ose*. The monosaccharides are classified as trioses, tetroses, pentoses, hexoses, etc., by the number of carbon atoms in the molecule. Each class is further subdivided, according as it possesses an aldehydic or ketonic radical, into aldoses and ketoses. In some sugars the primary alcohol group at the end of the carbon chain is reduced to a methyl group—rhamnose, fucose.

The natural sugars are optically active: they contain several asymmetric carbon atoms, so that the number of possible stereoisomerides is considerable. Comparatively few of these possibilities have been realised in nature, but nearly all those predicted by stereochemical theory have been produced synthetically. This achievement, largely the work of Emil Fischer, is not a recent one, but its story still affords one of the most fascinating chapters in

organic chemistry. The successful solution of so complex a problem gave a great incentive to subsequent work which is elucidating the structure of even more complex natural products. It has been estimated that three-quarters of the dry weight of the plant world is made up of carbohydrates, so that the natural limitation of the hexoses to four indicates a selective process during the period of organic evolution.

In the following account glucose will be taken as the typical sugar, and its structure, properties and reactions will be considered, more particularly with reference to their biochemical importance. Questions involving the physical chemistry and the more obscure chemical reactions of glucose will receive only limited consideration.

Many hypotheses have been discarded during the evolution of the present views of the structure of the sugars. It is the writers' aim to present the present position in the simplest terms and as a logical whole, but they will indicate alternative opinions if only for the reason that it cannot be considered that finality has yet been attained on certain topics. The chief utility of any hypothesis is to stimulate further progress.

## Glucose.

Glucose is also known as dextro-glucose, abbreviated to dextrose to express the sign of its optical activity, and as grape sugar on account of its occurrence in the juice of the grape. It is formed from many of the more complex carbohydrates on hydrolysis either by means of enzymes or acids. It is easily prepared from starch in commerce and can be purchased in a state of high purity at a low cost.

*Constitution.*—Glucose has the molecular formula $C_6H_{12}O_6$.

On oxidation it yields gluconic acid, a monobasic acid $C_5H_{11}O_5 . COOH$. Reduction with sodium amalgam gives the hexahydric alcohol, sorbitol $C_6H_{14}O_6$: reduction with hydrogen iodide gives *n*-hexyl iodide, proving that it has a straight chain of carbons. It forms pentacetates, pentabenzoates, etc. Many class reactions of aldehydes are shown; it reacts with phenyl hydrazine and hydroxylamine and adds hydrogen cyanide; one oxygen then exerts an aldehyde function.

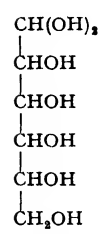
A constitutional formula may be advanced :—

$$CH_2(OH) . CH(OH) . CH(OH) . CH(OH) . CH(OH) . CHO \qquad \text{I.}$$

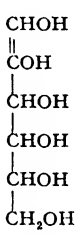
This formula I. was suggested by Baeyer [1] in 1870, and it formed the basis of Emil Fischer's more elaborate formula, in which the space position of each hydroxyl relative to the carbon chain was determined.

The alternative structure of an aldehydrol (II.) was proposed by Fittig,[2] and that of an ethylene oxide (IV.) by Colley,[3] as more in keeping with the relative suppression of the aldehydic properties of glucose.

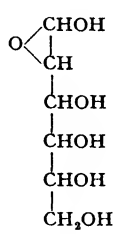
The historical development of structure was as follows :—



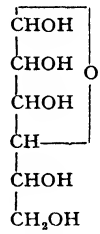
II.
Aldehydrol, Fittig, 1871.



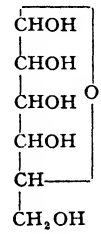
III.
Enol, Fischer, 1895.



IV.
Ethylene Oxide, Colley, 1870.

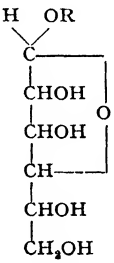


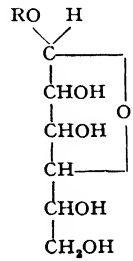
V.
Butylene Oxide, Tollens, 1883.



VI.
Amylene Oxide, Haworth, 1926.

It became necessary to explain, however, the existence of two well-defined series of isomeric glucose derivatives designated $\alpha$ and $\beta$ respectively, in which the isomerism could only be explained by differences in the relative positions of the groups attached to the potentially aldehydic carbon. Such difficulty was largely met by the formula V. which had been first suggested by Tollens [4] in 1883, in which four of the carbon atoms are included in a ring, together with a single oxygen atom. It contains one more asymmetric centre than the aldehyde formula, and so permits the existence of two series of derivatives :—





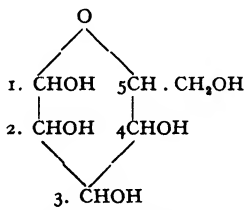
This ring was termed the butylene oxide or pentaphane ring, and was to a large extent based on an analogy with the formula of the lactone of gluconic acid; it was adopted by Fischer [5] for the methyl-glucosides in 1893. The formula came more and more into prominence when the relationship of the $\alpha$- and $\beta$-forms of glucose to the $\alpha$- and $\beta$-methylglucosides was established by Armstrong,[6] and indeed received general adoption, until the discovery that there existed yet

another series of isomeric glucose derivatives, caused the evidence on which it was based to come once more under review.

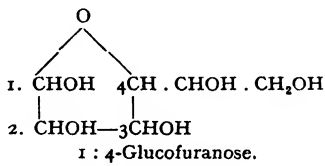
In the meantime Fischer [7] in 1895 had suggested that in some transformations, notably in presence of alkali, glucose reacted as an enol (III.).

Various alternative ring formulæ were suggested for the new glucose derivatives which were characterised by their relative instability, at first by Irvine, and later by Haworth, who set themselves the task of proving from first principles the structure of glucose, making use of the fact that all the hydroxyl groups other than that concerned in the oxygen ring could be masked by methylation. Avoiding details for the moment it may be stated that in 1926 Haworth and his collaborators [8] were able to afford conclusive proof that the normal $\alpha$- and $\beta$-glucoses and their derivatives contain a six-membered ring, in which carbons one and five are joined by the oxygen bridge and that formula VI. represents ordinary glucose. Haworth's further investigation showed that the less stable so-called $\gamma$-glucose derivatives contain the five-membered ring in which the oxygen bridge joins carbons 1 and 4, namely, formula V., first suggested by Tollens for ordinary glucose.

Haworth designates the two formulæ for glucose as 1 : 5-glucopyranose and 1 : 4-glucofuranose to emphasise their ring structure, and writes them with a primary alcohol — $CH_2OH$ side chain and a longer side chain —CH(OH) . $CH_2OH$ respectively in the following manner :—



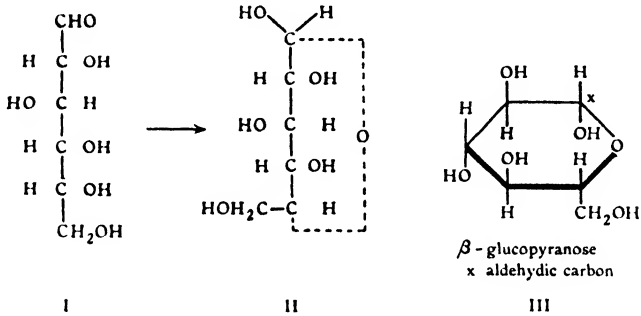

1 : 5-Glucopyranose.




1 : 4-Glucofuranose.

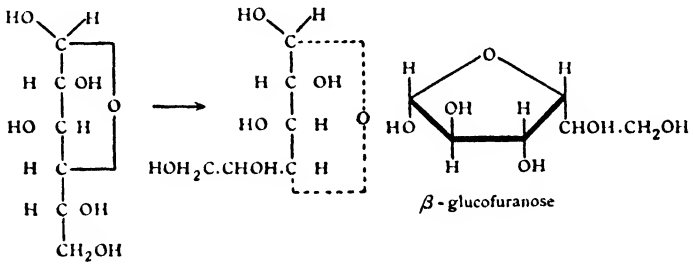
When one proceeds to indicate the spatial relationship of the various hydroxyl groups in the Haworth ring formulæ, a correction has to be made in the relative position of the groupings at $C_5$ in the Fischer configuration formula I. This is necessitated by the fact that the ring closure from an open chain to a cyclic form causes the rotation of the linking between $C_5$ and $C_6$ through 120° which has the effect of bringing the H at $C_5$ on the opposite side of the carbon chain. The configuration formula is thus more correctly represented by II.

Haworth introduced a perspective formula III. which is more satisfactory than II. and it is likely to enter into general use. It shows clearly the groups above and below the plane of the carbon ring:—




I II III

A corresponding alteration has obviously to be made in the formula of glucofuranose as shown below :—




In the following pages the alternative ways of writing these formulæ will be used in the form best suited to illustrate the particular point under discussion.

There has been much controversy aroused before these ring formulæ could be accepted as settled, which now, due to the contributions of a number of workers, appears to be satisfactorily achieved.

On the one hand, those who have used methylation methods have now furnished transformations which can be unequivocally interpreted, and are probably free from former objections based on the criticism that ring structure is altered on methylation. On the other hand, the evidence that Hudson had brought forward based on certain rules derived from the optical rotation constants of the sugars, which resulted in different conclusions as to ring structure from those arrived at by methylation methods, especially with mannose, has

been shown to be less weighty. This is because the present-day understanding of the theory underlying optical activity, owed to the work of Levene and of W. Kuhn and Freudenberg, has shown that the Van't Hoff law of optical superposition, on which Hudson based his reasoning, does not hold strictly for compounds such as the sugars, and therefore cannot be relied on absolutely for determining structure.

The interpretation of optical constants will be found discussed in Chapter IV.

The proof of the ring formulæ of normal and $\gamma$-glucose is based on the obtaining of fully methylated derivatives without causing a shift in ring structure, and a degradation of these fully methylated compounds by oxidation to substances of known structure, from which the position of the oxygen ring can be deduced. Crystalline glucose itself, as will be emphasised in later chapters, yields in solution an equilibrium containing a number of structurally different forms, and is therefore obviously unsuitable for direct methylation experiments. The $\alpha$- and $\beta$-methylglucosides of both normal and $\gamma$-glucose are much more stable substances. They do not show mutarotation and the $\alpha$- and $\beta$-forms are not easily interconvertible: nor do they undergo a change from one ring form to another.

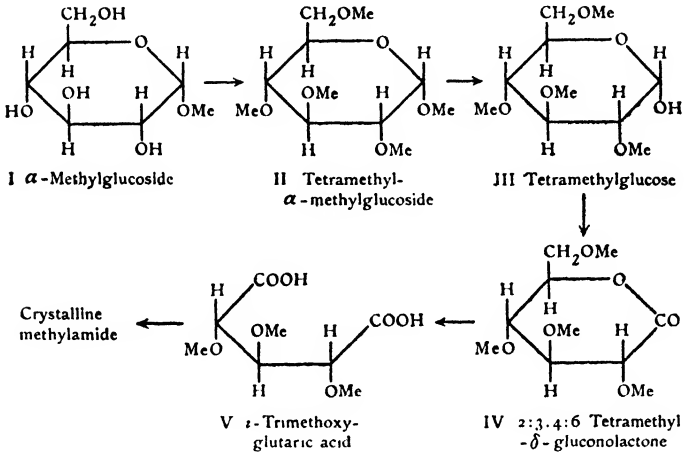
The work of E. F. Armstrong, referred to in detail on page 23, has proved that the normal $\alpha$- and $\beta$-methylglucosides have the same ring structure as normal $\alpha$- and $\beta$-glucose, a conclusion which is supported by a large amount of evidence from the physical properties.

A proof of the ring structure of the methylglucosides is therefore equivalent to the proof of the ring structure of the corresponding forms of glucose.

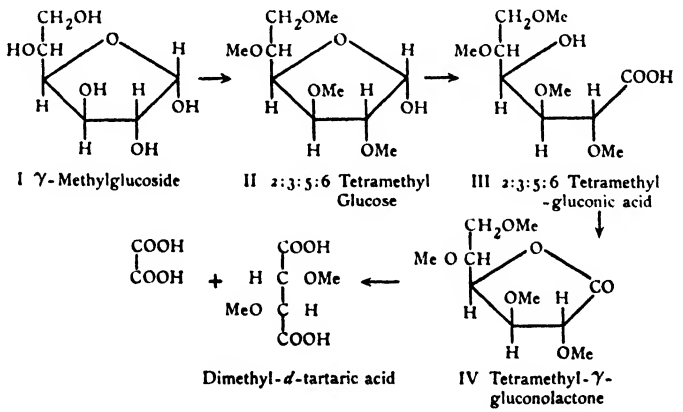
The assumption is made throughout that the more stable methylglucosides do not undergo any change in ring structure during methylation.

Two methylation methods are available: that of Purdie and Irvine,[9] by means of methyl iodide and silver oxide, and that of Haworth [10] involving digestion with methyl sulphate and alkali in aqueous solution. Both methods convert the $\alpha$- and $\beta$-methylglucosides (I.) into their tetramethyl derivatives (II.). Taking either of these and submitting it to acid hydrolysis the corresponding tetramethylglucose (III.) is obtained. Both of these, when oxidised by means of bromine water, give rise to the same tetramethyl-$\delta$-gluconolactone (IV.), which on further oxidation with nitric acid is converted into *i*-trimethoxy glutaric acid (V.). This could be identified beyond question through its

crystalline methylamide. The yields at each stage are almost quantitative.




The series of degradations shows definitely that the oxide ring must be attached either at the fifth or sixth carbon atom of the chain. There are a large number of observations to exclude the latter alternative. Firstly, methylglucoside is easily oxidised at position 6 to form the glycoside of glucuronic acid.[11] Secondly, 6-triphenylmethyl glucose yields a triacetate and this a triacetylglucose which passes to a methylpentose on reduction of the corresponding halogen derivative.[12] Thirdly, 2 : 3 : 4 : 5-tetramethyl-gluconic acid has also been prepared and shows no tendency to form a lactone, and is clearly different from 2 : 3 : 4 : 6-tetramethyl-gluconic acid which forms a lactone.



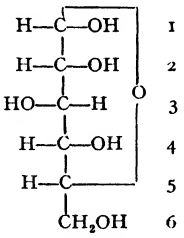
The proof of the structure of glucofuranose has been afforded in a similar manner. The methylation product of $\gamma$-methylglucoside (I.) is a liquid form of tetramethylglucose (II.).[13] It gives rise on oxidation to 2 : 3 : 5 : 6-tetramethyl-gluconic acid (III.) which forms a characteristic lactone (IV.). This, when digested with hot nitric acid gives rise to a mixture of dimethyl-*d*-tartaric acid and oxalic acid.

These observations show that scission of the carbon chain has occurred between the fourth and fifth carbon atoms. It follows that $\gamma$-methylglucoside has a five-atom ring structure.[14]

By these same methods Haworth and his co-workers have shown that the normal sugars of the hexose, pentose and methyl pentose series are all pyranoses; whereas the $\gamma$-sugars are all furanoses. Proofs for individual sugars will be discussed under the several sugars.

## Nomenclature.

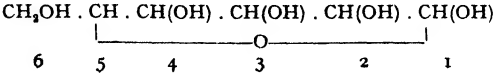
When dealing with a molecule capable of existence in so many stereoisomeric forms, it is necessary to adopt a precise nomenclature.




α-*d*-Glucose.

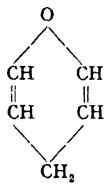
The carbon atoms are numbered as shown above, and in making reference to the groups attached to them it is convenient to use the abbreviation $C_1$, $C_2$, etc. The use of Greek prefixes is to be avoided for describing the carbons: it should be restricted to isomerides of a particular substance. In particular, the two alternative configurations of the groups on $C_1$ are designated by $\alpha$ and $\beta$ as in the methylglucosides.

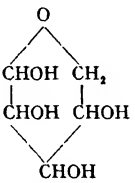
For the sake of uniformity it is desirable when writing the formula vertically to have the reactive, potentially aldehydic group upwards, and when writing it horizontally to have this group to the right—

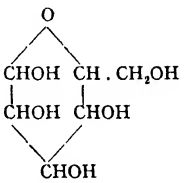


It has been mentioned that Haworth introduced the terms pyranose and furanose to describe the ring structures of normal and reactive glucose. The derivation of these terms is as follows :—

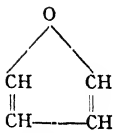
The simplest type of ring containing five atoms and one oxygen is pyran and the simplest sugar derived from it would be a pentose, tetrahydrotetrahydroxy-pyran or pyranose. The introduction of a side chain gives a typical aldohexose. Normal glucose is therefore glucopyranose.

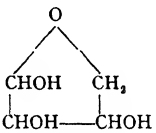

Pyran.

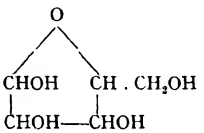

Pyranose.

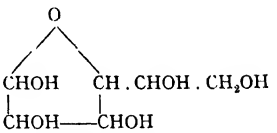

Glucopyranose.

Similarly, the simplest type of five-membered ring containing oxygen is furan, and the simplest type of furanose sugar is (ideally) a tetrose. On this system $\gamma$-glucose becomes glucofuranose.


Furan.


Furanose.


Arabofuranose.


Glucofuranose.

For greater clarity the points of attachment of the oxygen bridge are indicated by numerals. Thus normal methylglucoside is methyl glucopyranoside 1 : 5 and $\gamma$-methylglucoside is methyl glucofuranoside 1 : 4. By suitable distribution of the hydrogens and hydroxyls any of the known sugars can be represented.

The terms glucopyranose, glucofuranose, etc., are self-explanatory, and their adoption is recommended as overcoming previously confusing aspects of sugar nomenclature.

The prefix *epi* is used to denote the new carbohydrate formed by the interchange of the H and OH groups on carbon 2 : thus mannose is epiglucose, ribose is epiarabinose. The change is spoken of as epimerism, and the isomeric pair as epimerides.

The term methylglucose is used for all derivatives in which OH is substituted by $OCH_3$ : it is desirable for the avoidance of confusion to distinguish these O-methyl derivatives from the C-methyl derivatives, the methylpentoses in which the group on carbon 6 is $CH_3$

instead of $CH_2OH$. Professor Votoček has suggested the prefix methylo for the O-methylated sugars : it is worthy of consideration.

It may be helpful to indicate certain differences of nomenclature which are followed by writers in foreign journals. The French have adopted the recommendations of an International Commission for the Reform of Scientific Nomenclature. These recommendations have not found favour in English-speaking countries and are not accepted generally.

The term *glucide* is used for carbohydrates : it does not mean the same as the English glucoside. *Ose* signifies a monosaccharide, and *polyose* a polysaccharide : *glucose* or *glycose* is used as a general form for the sweet tasting monosaccharides. (Greek *glukus*—sweet.)

Sugar derivatives are divided into *hologlucosides* or *holosides*, comprising the mono-, di- and trisaccharides, and the *héteroglucosides* or *hétérosides*, compounds of carbohydrates with other organic substances, such as the glycosides and tannins.

In German publications the $\gamma$-forms of sugars are often referred to as *am*-sugars (alloiomorphic) or as *h*-sugars (hetero), in contrast to the normal *n*-sugars.

Freudenberg has introduced the convenient term *oligosaccharide* to include the di-, tri-, tetrasaccharides, etc., as a class distinct from the polysaccharides, which are comprised of a much greater number of simple units. We shall adopt it here.

## BIBLIOGRAPHY.

Owing to the immense number of publications on the chemistry of the sugars, the bibliography cannot claim to be to any degree complete, but an attempt has been made to provide references to the more important papers. The abbreviations used correspond to the practice of the Chemical Society, except that the shortened forms J.B.C. and J.A.C.S. have been used for the Journal of Biological Chemistry and the Journal of the American Chemical Society.

### TEXTBOOKS.

E. F. Armstrong and K. F. Armstrong, *The Glycosides*, London, 1931.
K. Bernhauer, *Chemie der Zuckerarten*, Berlin, 1933.
K. Bernhauer, *Oxydativen Gärungen*, Berlin, 1932.
E. Fischer, *Untersuchungen über Kohlenhydrate und Fermente*, I, 1884-1908, II, 1908-1919, Berlin, 1909 and 1922.
K. Freudenberg, *Tannin, Cellulose, Lignin*, Berlin, 1933.
A. W. van der Haar, *Anleitung zum Nachweis, zur Trennung und Bestimmung der Monosaccharide und Aldehydsäuren*, Berlin, 1920.
A. Harden, *Alcoholic Fermentation*, London, 1932.
W. N. Haworth, *The Constitution of Sugars*, London, 1929.
P. Karrer, *Polymere Kohlehydrate*, Leipzig, 1925.

W. KUHN AND K. FREUDENBERG, *Drehung der Polarisationsebene des Lichtes*. Band 8. Abschnitt III. Hand-und Jahrbuch der Chemischen Physik., Leipzig, 1932.
P. A. LEVENE AND W. BASS, *Nucleic Acids*, New York, 1931.
P. A. LEVENE, *The Hexosamines and Mucoproteins*, London, 1925.
E. VON LIPPMANN, *Die Chemie der Zuckerarten*, Braunschweig, 1904.
K. H. MEYER AND H. MARK, *Die Aufbau der Hochpolymeren Naturstoffe*, Leipzig, 1930.
H. PRINGSHEIM, *Polysaccharide*, Berlin, 1931.
H. PRINGSHEIM AND J. LEIBOWITZ in *Klein's Handbuch der Pflanzenanalyse*, Vienna, 1932.
J. VAN RIJN, *Die Glucoside*, Berlin, 1931.
B. TOLLENS, *Kurzes Handbuch der Kohlehydrate*, Leipzig, 1914.
VOGEL AND GEORG, *Tabellen der Zucker und ihrer Derivate*, Berlin, 1931.

## REFERENCES TO CHAPTER I.—THE STRUCTURE OF GLUCOSE.

1. BAEYER, Ber., 1870, **3**, 67.
2. FITTIG, Z. Rüb. Ind., 1871, **21**, 270.
3. COLLEY, Compt. rend., 1870, **70**, 703.
4. TOLLENS, Ber., 1883, **16**, 921.
5. FISCHER, Ber., 1893, **26**, 2400.
6. ARMSTRONG, J.C.S., 1903, 1305.
7. FISCHER, Ber., 1895, **28**, 1145.
8. CHARLTON, HAWORTH AND PEAT, J.C.S., 1926, 89.
9. PURDIE AND IRVINE, J.C.S., 1903, 1021.
10. HAWORTH, J.C.S., 1915, 8.
11. SMOLENSKI, Rocz. Chem., 1923, **3**, 153.
12. HELFERICH, KLEIN AND SCHÄFER, Ann., 1926, **447**, 19.
13. IRVINE, FYFE AND HOGG, J.C.S., 1915, 524.
14. HAWORTH, HIRST AND MILLER, J.C.S., 1927, 2436.

## CHAPTER II.

### STEREOISOMERISM OF THE ALDOSES.

A COMPOUND represented by the empirical formula,

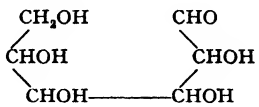
$$CH_2(OH) . CH(OH) . CH(OH) . CH(OH) . CH(OH) . CHO,$$

containing four asymmetric carbon atoms, should, according to the Le Bel-Van't Hoff theory, be capable of existing in sixteen stereoisomeric forms, eight of which would be mirror images of the other eight and of equal but opposite rotatory power.

Thus, corresponding to ordinary dextro-glucose (*d*-glucose), there is a lævorotatory isomeride *l*-glucose of equal and opposite rotatory power, of like configuration but being its mirror image. In fact, when glucose is prepared by artificial means from optically inactive material, a mixture in equal proportions of *d*- and *l*-forms is always obtained. Such a mixture is optically inactive.

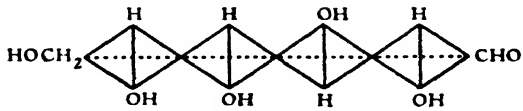
Although only four aldohexoses occur naturally (glucose, mannose, *d*- and *l*-galactose), all of the sixteen possible isomerides are now known. Emil Fischer, to whom we owe the discovery of this remarkable series, not only showed how they may be prepared, but made many of them in such ways that their configuration is established.

In the projection formula the aldehyde sugar is represented with the carbons lying in a straight line, although because of the tetrahedral angle between the valencies the molecule is more accurately represented in a ring form :—



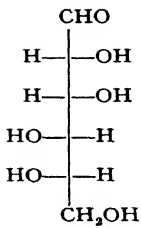

It is important to avoid representing the projection formula as a zig-zag chain.

The projection formula can best be illustrated on paper by representing the four centre carbons with tetrahedra :—

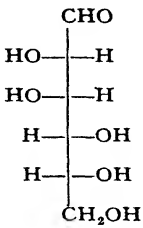



*d*-Glucose.

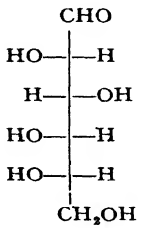
ALDOHEXOSES.



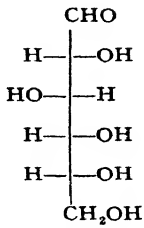

*l*-Mannose.



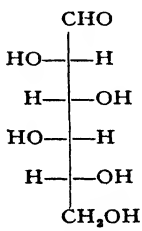

*d*-Mannose.



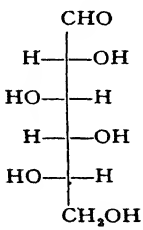

*l*-Glucose.



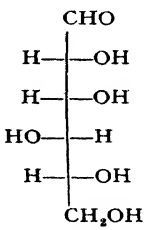

*d*-Glucose.



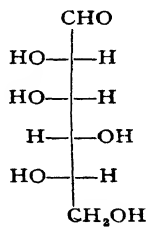

*d*-Idose.



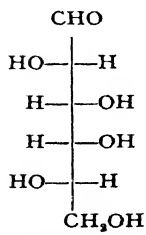

*l*-Idose.



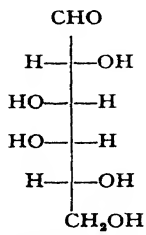

*d*-Gulose.



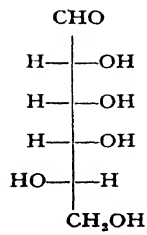

*l*-Gulose.



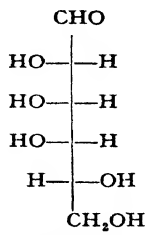

*l*-Galactose.



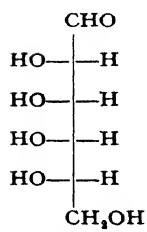

*d*-Galactose.



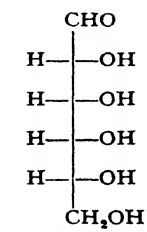

*l*-Talose.



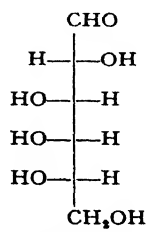

*d*-Talose.



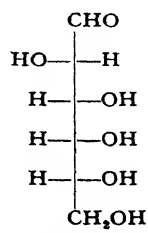

*l*-Allose.




*d*-Allose.



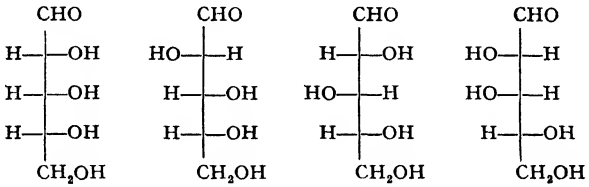

*l*-Altrose.




*d*-Altrose.

It is desirable to indicate briefly the manner in which the configuration of the sugars has been determined, as the same methods serve for new compounds which may be found to occur naturally or may be synthesised in the laboratory.

The most straightforward method of procedure is to determine first the structure of the pentoses and from them that of hexoses.




There are eight possible aldopentoses, that is, four pairs of optical antipodes, and considering only the *d*-forms, there are four alternative formulæ.

The relevant facts are:—

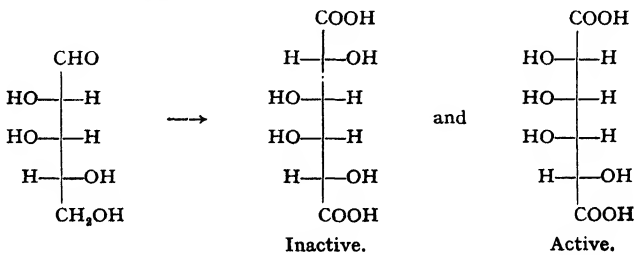
(1) Arabinose and ribose give the same osazone with phenyl hydrazine, hence their configuration must be identical except as regards $C_2$. Arabinose and ribose must be (1 and 2) or (3 and 4).

(2) On oxidation arabinose gives an optically active dibasic acid; ribose and xylose give optically inactive dibasic acids. Pentoses 2 and 4 will give an optically active dibasic acid, from 1 and 3 the acids will be optically inactive.

Hence arabinose is either 2 or 4, ribose and xylose are 1 and 3, lyxose is either 4 or 2.

(3) When hydrogen cyanide is added to the pentose and a new asymmetric carbon atom introduced, and the compound is subsequently oxidised to a dibasic acid, it is found that arabinose gives a mixture of two acids, both of which are optically active, whereas lyxose gives a mixture of two acids, one active and one inactive.

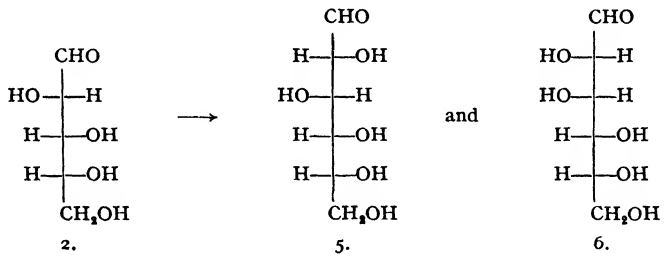
This can only happen with 4:—



Accordingly lyxose has the constitution 4, so that arabinose is 2, ribose 1, and by elimination xylose is 3.

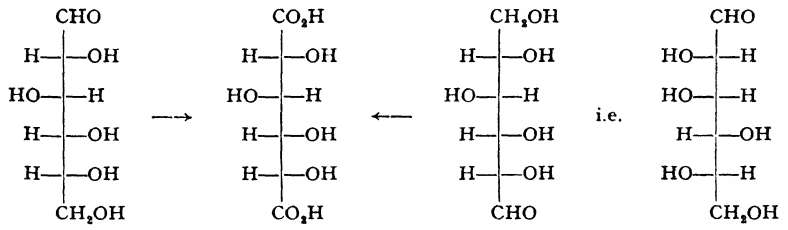
Proceeding from the pentoses to establish the formula of the hexoses we have—

(1) Arabinose gives rise by Kiliani's reaction (addition of hydrogen cyanide) to two hexoses, glucose and mannose :—

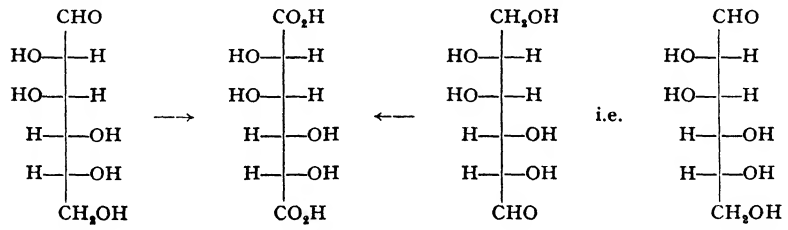



Hence glucose must be either 5 or 6.

(2) The same dibasic acid is produced on oxidation of glucose as from another hexose, gulose, viz. saccharic acid. This means that the configuration of each of the four asymmetric carbon atoms is the same and that, therefore, the difference between the two sugars is that their primary alcohol ($CH_2OH$) and aldehydic groups are interchanged :—




From 5 a new sugar 7 is formed by this process—



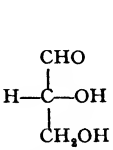

whereas from 6 the same sugar 8 is formed.

Accordingly, glucose is represented by formula 5, mannose by formula 6, and gulose by formula 7. An extension of the reasoning leads to the formulæ for the other hexoses.

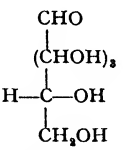
In sugar chemistry the prefixes *d* and *l* have a special meaning in that they indicate stereochemical relationships rather than the sign of the optical rotation. In other fields the prefix usually denotes merely the sign of rotation, for example, dextrorotatory *d*-mandelic acid forms lævorotatory *l*-mandelonitrile.

The formulæ assigned to *d*- and *l*-glucose are chosen arbitrarily; that is to say, it is assumed that in the *d*-form the groups occupy a certain position, whence it follows that in the stereoisomeride they are present in the reversed position. For the original proof of the validity of the formulæ and the arguments by which they are deduced, the reader is referred to Fischer's [1] classical summary. A further convention is to indicate as belonging to the *d*-series all compounds derived from dextro-glucose by simple reactions which leave the absolute configuration of the molecule unchanged. In many instances, as for example, *d*-fructose and *d*-arabinose, the new compound rotates polarised light to the left, so that the prefix does not give a correct indication of the sign of the rotation. Similarly, all compounds derived from lævo-glucose are designated as of the *l*-series though they may be dextrorotatory.

Wohl [2] was able to establish that dextrorotatory *d*-glycerose, the simplest optically active sugar of the *d*-series, possesses the same absolute configuration for its asymmetric carbon as for the corresponding $C_5$ of *d*-glucose.




*d*-Glycerose.




*d*-Glucose.

Based on *d*-glycerose, therefore, the same classification was obtained as that derived by Fischer based on *d*-glucose. Four compounds, gulose, idose, xylose and threose were originally described by Fischer with the prefix *d*, their relation to *d*-glucose being based on the gulose actually obtained by Fischer in practice, by an operation equivalent to turning round the asymmetric system of glucose, which in the light of our present knowledge obviously involves passing from the *d* to the *l* series; they should properly be regarded as *l*. Their *l*-isomerides hence become *d* and are incorporated in the

adjoining table as such. The natural compound in early days described as *l*-xylose in the literature is properly *d*-xylose and its relation to glucose is made much clearer when it is so named.

To avoid ambiguity, sugars can be labelled so as to show both their stereochemical relationships and the sign of the optical rotation, by using the prefix *d* or *l* for the former and the signs + or — for the latter. Thus one has *d*(+) glucose, *d*(—) fructose, *d*(—) xylose, etc.

A useful suggestion for the simplification of the symbols showing the steric relationships of the sugars was made by Wohl.[3] Instead of writing the whole formula vertically attention is confined to the H and OH groups on one side of the molecule (the right) only, and these are written down in order. If it is agreed to consider the aldehyde group as being to the right of the formula when written horizontally, and the groups are below the line, *d*-glycerose becomes OH, *d*-glucose is OH OH H OH, and *l*-glucose is H H OH H. An alternative suggestion by Wohl is to write *d* for each OH below the carbon chain and *l* for each H. Accordingly, glucose is *ddld* aldohexose,

| Triose. | Tetroses. | Pentoses. | Hexoses. |
|---|---|---|---|
| OH (+) *d*-Glycerose. | OH OH (+ +) *d*-Erythrose. | OH OH OH (+ + +) *d*-Ribose. | OH OH OH OH (+ + + +) *d*-Allose. |
| | | | OH OH OH H (+ + + −) *d*-Altrose. |
| | | OH OH H (+ + −) *d*-Arabinose. | OH OH H OH (+ + − +) *d*-Glucose. |
| | | | OH OH H H (+ + − −) *d*-Mannose. |
| | OH H (+ −) *d*-Threose. | OH H OH (+ − +) *d*-Xylose. | OH H OH OH (+ − + +) *d*-Gulose. |
| | | | OH H OH H (+ − + −) *d*-Idose. |
| | | OH H H (+ − −) *d*-Lyxose. | OH H H OH (+ − − +) *d*-Galactose. |
| | | | OH H H H (+ − − −) *d*-Talose. |

and symbols can be given to the sugars which avoid the use of OH and H. Fischer, following Van't Hoff, originally used + and − to denote the position of these groups.

The table on p. 17 shows all the possible tetrose, pentose and hexose sugars derived from *d*-glycerose, i.e. *the sugars of the* d-*series*, according to both Fischer's and Wohl's symbols.

The optical antipodes of these sugars—the *l*-series—are derived from *l*-glycerose or *l*-glucose and can be incorporated in a similar table.

The *d* and *l* nomenclature of the sugars is thus based on their relationship to *d* and *l*-glycerose and is irrespective of the sign of their optical rotatory power.

The derivation of absolute configuration of substances related to the sugars has been achieved in many instances in recent years, in particular by Levene [4] and by Freudenberg.[5]

Taking *d*-glycerose as a *d*-compound for reference, it has been shown that the following natural products have the configurations indicated by their prefixes, *l*(+) lactic acid, *l*(−) malic acid, *d*(+) tartaric acid, and that all the natural amino acids, irrespective of the sign of rotation, belong to the *l*-series. The details of the methods whereby these configurations have been established are, however, outside the scope of this monograph.

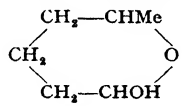
## REFERENCES TO CHAPTER II.—STEREOISOMERISM OF THE ALDOSES.

1. Fischer, Ber., 1894, **27**, 3189.
2. Wohl and Neuberg, Ber., 1900, **33**, 3095. Wohl and Momber, Ber., 1917, **50**, 455.
3. Wohl and Schellenberg, Ber., 1922, **55**, 1404. Wohl and Freudenberg, Ber., 1923, **56**, 309.
4. Levene, Chemical Reviews, 1925, **2**, 179.
5. Freudenberg, Ber., 1914, **47**, 2027.

## CHAPTER III.

### GLUCOSE AS PYRANOSE, FURANOSE AND ALDEHYDE.

THE ring formulæ of the carbohydrates constitutes merely a special case of the tautomerism between open chain hydroxyaldehyde and cyclic semiacetal forms exhibited by the simple hydroxyaldehydes investigated by Helferich,[1] which exist in both open chain and cyclic forms. Thus δ-hydroxy hexaldehyde is stable in the cyclic form and shows many analogies with reducing sugars.



In a solution of glucose which has reached equilibrium, it is theoretically possible for all the different oxide ring forms to be present, even if some are in extremely small proportions, in addition to the free aldehyde form : thus there may be present—

Free aldehyde.
3 ring = α-ethylene oxide.
4 ring = β-propylene oxide.
5 ring = γ-butylene oxide, i.e. furanose.
6 ring = δ-amylene oxide, i.e. pyranose.
7 ring = ε-hexylene oxide.

Each ring form would occur in α and β modifications. The relative proportions of the different isomers found in the equilibrium would be determined by their relative thermodynamic stability, and stereochemical considerations here naturally favour the formation of the 6- and 5-membered pyranose and furanose rings. The presence of other tautomeric modifications, such as the 1 : 2 dienol suggested by Fischer, should not be left out of consideration.

Different reagents react at different rates with different isomers, and the composition and the structure of the product depends on these rates and upon the rate of conversion of one isomer into another, under the particular experimental conditions chosen.

The conditions used may even be such as to furnish as a product a substance whose structure is only represented in the original solution

as a minor component. By taking advantage of these facts, it is possible to prepare and isolate as crystalline substances derivatives of many of the theoretically possible isomeric forms.

The presence of acids favours the equilibrium in the direction of the furanose forms, or at any rate the production of furanose derivatives. Isbell [2] found that the equilibrium between the various forms of gulose is altered by the addition of calcium chloride, and α- and β-gulose and α- and β-methyl gulosides can be isolated and purified through their crystalline calcium chloride addition products.

Mannose [3] yields a crystalline compound *d*-mannose, $CaCl_2$, $4H_2O$, which has been shown by Isbell [4] to be a derivative of mannofuranose : this derivative is obtained in spite of the fact that an aqueous solution of mannose contains predominantly the pyranose form.

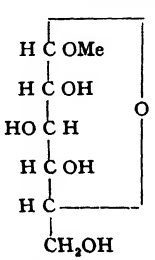
Purely chemical means alone do not allow one to ascertain the relative proportions of the different isomers in an aqueous solution of glucose, and only give incomplete information as to their nature.

It has often been supposed that forms of glucose are present in solution in the equilibrium mixture, perhaps in very small amounts, which are very reactive and take part in vital reactions, and that catalysts present in living cells promote rapid re-establishment of the equilibrium, so providing a fresh supply of reactive isomer.

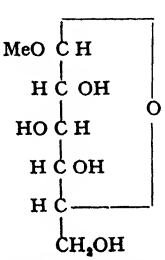
This has caused the conception of " active glucose " as a reactive structural isomer, not a thermally activated ordinary glucose molecule, and has been identified by various workers with one or other of the theoretically possible static modifications of glucose such as gluco-furanose. The subject of " active glucose " will be reconsidered later : it is a very important problem for those who are interested in vital syntheses.

## Glucopyranose Derivatives.

α- and β-methylglucosides 1 : 5.




α-Methylglucoside.




β-Methylglucoside.

Two of the most important derivatives of glucose are the two isomeric methylglucosides (α and β) which are formed by the interaction of glucose and methyl alcohol. They are the prototypes of the natural glucosides which, however, are, in fact, all β-glucosides. Emil Fischer [5] first discovered them in 1893.

Both are formed when a solution of glucose in cold methyl alcohol is saturated with dry hydrogen chloride gas. The solution in time loses all cupric-reducing power. After neutralising with lead carbonate and concentration the α-methylglucoside crystallises, while the β-methylglucoside can be isolated later from the mother liquor and was first so obtained crystalline by Van Ekenstein.[6] A better method [7] is to boil the sugar in a 0·25 per cent. alcoholic hydrogen chloride solution. β-methylglucoside is obtained when glucose is carefully methylated by means of dimethyl sulphate and sodium hydroxide.[8]

Returning to the preparation of the glucosides just described, it will be noted that both forms are produced simultaneously, the α-isomeride predominating. When solid anhydrous glucose (α-glucose) is dissolved in dry methyl alcohol containing dry hydrogen chloride, the first change is its rapid conversion into a mixture of α- and β-glucose in nearly equal parts. Each of these then undergoes etherification, the principal product being a mixture of α- and β-methylglucosides, in which the latter is slightly in excess. On standing, slow conversion of the β-methylglucoside into the more stable α-isomeride takes place. The equilibrated mixture of the glucosides contains 77 per cent. of the α- and 23 per cent. of the β-isomeride. If, however, the solution be neutralised as soon as etherification is complete and before the isomeric changes take place, and the solvent be removed, a mixture of the two glucosides in approximately equal quantities is obtained. These may be separated by fractional crystallisation.

Such a process is somewhat tedious when β-methylglucoside is the object of the preparation, and it is more convenient to make use of biological methods. On treatment with yeast, which contains the enzyme maltase, the α-methylglucoside is hydrolysed to glucose and methyl alcohol, and the glucose is removed by fermentation, so that β-methylglucoside, which is not attacked by yeast, alone remains, and can be isolated and purified.

When, on the other hand, α-methylglucoside is desired, the action of the acid is allowed to continue until equilibrium is attained, and, after crystallisation of some quantity of the α-methylglucoside, the

mother liquors are again heated with a little acid. This has the effect of causing the β-glucoside present to be converted into α-glucoside until equilibrium is again reached, when 77 per cent. of the total solid present is α-glucoside, and in consequence a further quantity of α-glucoside crystallises on removal of the solvent.

The methylglucosides differ considerably in their properties from glucose. They never behave as aldehydes, their optical rotatory power in solution remains unaltered on keeping. They do not interact with phenylhydrazine, and oxidising agents such as Fehling's solution are without action. They are hydrolysed by acids to methyl alcohol and glucose, the β-compound being attacked more rapidly than the α-form.

The methylglucosides are also hydrolysed by enzymes, but both isomerides are not hydrolysed by the same enzyme. In fact, the action of enzymes towards the glucosides is specific, and each form requires its own particular enzyme: α-methylglucoside is hydrolysed by maltase: β-methylglucoside by emulsin. The enzymes act at ordinary temperatures, preferably not above 37° C., and are far more active as hydrolytic agents than acids.

The methylglucosides are both colourless crystalline substances, the α-isomeride crystallising usually in long needles, the β-isomeride in rectangular prisms. Their physical properties, together with those of the corresponding ethylglucosides, are as follows:—

| | M. pt. °C. | $[\alpha]_D$. | Molecular Refractivity. |
|---|---|---|---|
| α-methylglucoside . . . . | 165-166° | + 159° | 70·17 |
| β-methylglucoside . . . . | 105° | − 34° | 70·55 |
| α-ethylglucoside . . . . | 113-114° | + 150·6° | |
| β-ethylglucoside . . . . | 73° | − 36·5° | |

The two glucosides are distinguished by the arbitrary prefixes α and β and the use of these prefixes at one time extended throughout the series of glucose derivatives, so that the prefix α indicated configurational identity with α-methylglucoside, until the discovery of substitution reactions in which a Walden inversion took place at carbon 1 made it difficult to establish the configuration always with certainty. Consequently, Hudson's nomenclature (see p. 36), based on the relative rotatory powers of an isomeric pair, the most dextrorotatory being called α, has been adopted, with the result that different α-derivatives of a sugar do not necessarily have the same absolute configuration.

## α- and β-Glucopyranose.

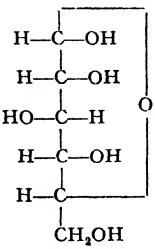
The two glucoses correspond in structure to the α- and β-methylglucosides.

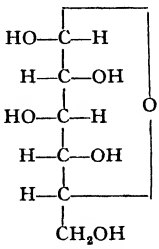
It was first shown by Tanret [9] in 1896 that besides the anhydrous and hydrated forms of glucose other isomeric anhydrous modifications could be obtained. He described an α-glucose $[\alpha]_D + 110°$, the initial rotatory power of which fell gradually to $[\alpha]_D + 52{\cdot}5°$; further, a β-glucose* of low initial rotatory power $[\alpha]_D + 19°$, increasing to $[\alpha]_D + 52{\cdot}5°$ in solution; and lastly, a glucose $[\alpha]_D + 52{\cdot}5°$ of unalterable rotatory power in solution. The three supposed isomerides were isolated by allowing glucose solutions to crystallise under different conditions; α-glucose separated at ordinary temperatures from solutions in 70 per cent. alcohol, and β-glucose from aqueous solutions at temperatures above 98°; the third form of glucose was obtained by precipitating a concentrated aqueous solution of glucose with alcohol. α-glucose hydrate crystallises from aqueous solutions at the ordinary temperature. When powdered anhydrous glucose is added to water, it immediately undergoes hydration before passing into solution.

A little later Simon [10] drew attention to the optical behaviour of the glucoses in relation to that of the methylglucosides of which the structure was known, and suggested that the glucoses were homologues of the glucosides and that both contained a closed ring with an oxygen bridge (see p. 30). Direct proof of this hypothesis was afforded by E. F. Armstrong,[11] who prepared the glucoses direct from the glucosides. Both glucosides are resolved into methyl alcohol and glucose by the appropriate enzymes, and as the enzymes effect the hydrolysis more quickly than the glucose which is formed can undergo isomeric change, it is possible to determine the nature of the sugar which is formed initially. In practice, this is done by preparing a clear solution of glucoside and enzyme, allowing hydrolysis to proceed for a short time, and then observing the rotatory power of the solution before and after the addition of a drop of ammonia which has the effect of establishing equilibrium immediately. A glucose of high initial rotatory power was obtained from α-methylglucoside by means of maltase, and one of low initial power from β-methylglucoside by means of emulsin. It is clear, therefore, that α- and β-

* Tanret's terms have been altered to bring them into agreement with the nomenclature now adopted.

glucose correspond respectively to the α- and β-glucosides and that they have the formulæ—


α-Glucose.


β-Glucose.

The change in rotatory power of freshly prepared solutions of glucose was shown to be a process of reversible isomeric change by Lowry [12] in 1899. Subsequently [13] he concluded that not only are α- and β-glucose isodynamic compounds but that Tanret's third form of glucose was a mixture in which these two and perhaps other compounds are present in equilibrium.

The configuration of the groups attached to carbon 1 in the isomeric glucoses has been established in several ways.

Böeseken [14] has established that for an alcohol to increase the electrical conductivity of boric acid solutions it must possess at least two hydroxyl groups, attached to two adjacent carbon atoms and situated on the same side of the carbon chain. Now the conductivity of boric acid in presence of α-glucose decreases during mutarotation as it is converted in part into β-glucose, whilst with β-glucose it increases; the change in conductivity takes place with the same velocity as the mutarotation, showing the two phenomena to be concurrent. Accordingly, α-glucose is assigned the formula in which the hydroxyl groups attached to carbons 1 and 2 are on the same side of the chain.

Riiber [15] finds that the molecular refractivity for D-light of α-glucose = 62·68 is lower than that of β-glucose = 63·07, in agreement with the general rule due to von Auwers that compounds containing two neighbouring hydroxyl groups in the *cis* position have a lower refractivity than the corresponding *trans* form.

Michaelis [16] in the methylglucosides found a higher dissociation constant for the β-form ($K = 2{\cdot}64 \times 10^{-14}$) than for the α-form ($K = 1{\cdot}97 \times 10^{-14}$), indicating an increase in acidity due to the greater separation of the two groups OH and $OCH_3$ in the β-isomeride.

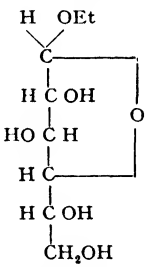
On the more chemical side the formulæ given are in harmony

with the facts established by Pictet [17] that α-glucose gives rise to α-glucosan (p. 113) to which an ethylene oxide structure is attributed for other reasons, whereas β-glucose gives rise to lævo or β-glucosan (p. 112) of which the structure is also known.

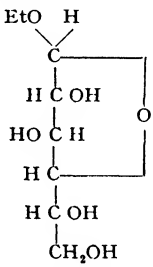
Consistent results have been obtained in connecting the reactivity of a number of glucosylhalides towards trimethylamine with the *cis* or *trans* configuration of the groups on $C_1$ and $C_2$.[18] A quaternary salt can only be formed with a *cis* configuration of these groups.

## Glucofuranose Derivatives.

Methyl or ethyl glucosides 1 : 4.



α-Ethylglucofuranoside.

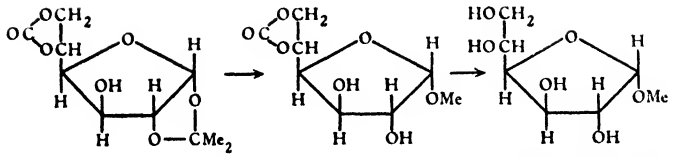


β-Ethylglucofuranoside.

Fischer originally showed that the reaction between glucose and methyl alcohol containing 1 per cent. of hydrogen chloride at the ordinary temperatures yielded a considerable amount of a syrup in addition to the two crystalline methylglucosides. He regarded this at the time as glucose dimethylacetal, but on reinvestigating the product twenty years later,[19] it was found to be a third isomeric methylglucoside which has since been designated as γ-methylglucoside. It is stable to alkalis, Fehling's solution and hot water, is hydrolysed by acids but not attacked by emulsin or by maltase.

Irvine, Fyfe and Hogg [20] have shown that this methylglucoside is a mixture of isomerides derived from an entirely new variety of glucose. This new methylglucoside is characterised by the remarkable ease with which it enters into condensation with acetone.

The labile γ-glucoses themselves have not been obtained crystalline, but Haworth, Porter and Waine [21] have prepared α-methylglucofuranoside in crystalline condition by forming glucofuranose-acetone carbonate which gives with methyl-alcoholic hydrogen chloride a crystalline α-methylglucofuranoside carbonate, which on treatment with baryta yields crystalline α-methylglucofuranoside $[\alpha]_D + 118°$.



α-methylglucofuranoside

Crystalline furanosides are reasonably stable to neutral aqueous permanganate, and the instability to this reagent attributed to furanosides is due to unsaturated impurities such as derivatives of furfuraldehyde to which furanosides are prone to give rise.

Goodyear and Haworth [22] prepared both α- and β-ethylglucofuranosides by the above-described method sometime previously. They are quite distinct from the ethylglucopyranosides.

| Ethylglucofuranosides. | M. pt. | $[\alpha]_D$. |
|---|---|---|
| α-ethylglucoside 1 : 4 . . . . | 82-83° | 101° |
| β-ethylglucoside 1 : 4 . . . . | 59-60° | −86° |

Among other crystalline furanose derivatives are the α- and β-forms of pentabenzoylglucofuranose [23] $[\alpha]_D + 58{\cdot}6°$ and $-52{\cdot}6°$. From the specific rotations of their derivatives, the rotations of the free sugars may be estimated, the α having a smaller dextrorotation than the α-glucopyranose and the β being strongly lævorotatory.

## Aldehydoglucose.

Sugar solutions show marked reduction of aldehyde reactivity; although they form oximes and hydrazones and reduce Fehling's solution, they do not form bisulphite addition compounds with readiness, nor do they give the test with Schiff's reagent.[24]

This lessened activity shows that the amount of free aldehyde present in solution must be small.

Attempts have been made to obtain evidence for the aldehyde form spectroscopically. At first it seemed that mono- and disaccharides with the exception of sucrose showed faint absorption at 2800 Å.U. characteristic of the carbonyl group.[25] Henri and Schou [26] and also Fischler [27] and Schlubach [28] came to the conclusion that in pure glucose solutions there was no carbonyl absorption. The absorbing impurity is probably methyl glyoxal. The method is sensitive to 0·3 per cent. of the aldehyde form, and it may therefore be said that there is less than this amount of free aldehyde present, if any.

The aldoses and ketoses all react with hydrogen cyanide to form

cyanhydrins. The amount of hydrogen cyanide bound in a given time by a freshly prepared solution of, say, α-glucose is less than that bound by a glucose solution which has reached the mutarotation equilibrium.

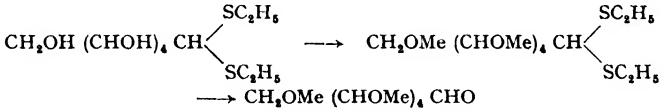
On the assumption that the free aldehyde form of a sugar is that which rapidly reacts with hydrogen cyanide, attempts have been made by Lippich [29] to estimate the amount of free aldehyde form present in equilibrated sugar solutions by measurement of the quantity of hydrogen cyanide bound. Different sugars give different values, and it is estimated that in the equilibrated solution glucose contains 0·25 per cent. aldehyde, galactose about 1 per cent. and mannose nearly 2 per cent. While the theoretical interpretation of these results is open to objections, the results are probably qualitatively correct. Glucose appears to contain less of the aldehyde form in equilibrium than any other sugar.

Galactose, mannose and rhamnose are said to redden fuchsine in aqueous solution, in opposition to glucose.

By indirect methods it has been possible to prepare derivatives in which the aldehyde group is free, but this constitutes no proof that aldehydo-glucose exists in ordinary glucose solutions.

Compounds proved to have an aldehydic structure are the pentamethyl derivatives of glucose, mannose and galactose.[30]

These were prepared by first making the glucose diethyl mercaptan, converting this to the pentamethyl derivative with dimethyl sulphate and then removing the mercaptan groups with mercuric chloride.



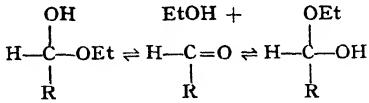

Pentamethyl aldehydoglucose forms a dimethyl acetal readily.

Likewise pentacetylglucose and galactose and tetracetylarabinose and xylose were synthesised from the corresponding dimethyl-mercaptals [31] and again aldehydopentabenzoylglucose.[32]

Apparently also galactose phenylhydrazone is a derivative of the free aldehyde form, since after acetylation the product is identical with that obtained from aldehydopentacetylgalactose and phenylhydrazine.

Aldehydopentacetylgalactose forms crystalline compounds with water and with ethyl alcohol which exhibit mutarotation in chloro-

form.[33] In the alcoholate there is evidence for the presence in solution of two semi-acetals in equilibrium with the aldehyde.




REFERENCES TO CHAPTER III.—GLUCOSE AS PYRANOSE, FURANOSE AND ALDEHYDE.

1. Helferich and Köster, Ber., 1923, **56**, 2088. Helferich and Fries, Ber., 1925, **58**, 1246.
2. Isbell, Bur. Stan. J. Res., 1930, **5**, 748.
3. Dale, J.A.C.S., 1929, 2788.
4. Isbell, J.A.C.S., 1933, 2166.

GLUCOPYRANOSE.

5. Fischer, Ber., 1893, **26**, 2400.
6. Van Ekenstein, Rec. trav. chim., 1894, **13**, 183.
7. Fischer, Ber., 1895, **28**, 1145.
8. Maquenne, Bull. Soc. chim., 1905, **33**, 260, 469. Schlubach and Maurer, Ber., 1924, **57**, 1686.
9. Tanret, Bull. Soc. chim., 1895, **13**, 728; 1896, **15**, 359.
10. Simon, Compt. rend., 1901, **132**, 487.
11. Armstrong, J.C.S., 1903, 1305.
12. Lowry, J.C.S., 1899, 213.
13. Lowry, J.C.S., 1903, 1314.
14. Böeseken, Ber., 1913, **46**, 2612; Rec. trav. chim., 1921, **40**, 354, 553.
15. Riiber, Norske Videnskabsselskab, 1931, **4**, 157.
16. Michaelis, Ber., 1913, **46**, 3683.
17. Pictet, Helv. Chim. Acta, 1920, **3**, 645.
18. Micheel and Micheel, Ber., 1930, **63**, 386.

GLUCOFURANOSE.

19. Fischer, Ber., 1914, **47**, 1980.
20. Irvine, Fyfe and Hogg, J.C.S., 1915, 524.
21. Haworth, Porter and Waine, J.C.S., 1932, 2254.
22. Goodyear and Haworth, J.C.S., 1927, 3136.
23. Schlubach and Huntenberg, Ber., 1927, **60**, 1487.

ALDEHYDOGLUCOSE.

24. Rayman, Ber., 1888, **21**, 2841.
25. Purvis, J.C.S., 1923, 2519.
26. Henri and Schou, Z. Physiol. Chem., 1928, **174**, 295.
27. Fischler, Biochem. Z., 1930, **227**, 156.
28. Schlubach, Z. Physiol. Chem., 1930, **186**, 148.
29. Lippich, Biochem. Z., 1932, **248**, 280.
30. Levene and Meyer, J.B.C., 1926, **69**, 175.
31. Wolfrom, J.A.C.S., 1929, 2188; J.A.C.S., 1930, 2464. Wolfrom and Newlin, J.A.C.S., 1930, 3619. Wolfrom and Morgan, J.A.C.S., 1931, 3390.
32. Brigl and Mühlschlegel, Ber., 1930, **63**, 1551.
33. Wolfrom, J.A.C.S., 1931, 2275.

## CHAPTER IV.

### THE SUGARS IN SOLUTION.

## Mutarotation.

It has long been known in the sugar group that the optical rotatory power of a solution of a freshly dissolved sugar changes gradually, increasing or falling until a constant value is reached. The change takes place very slowly when highly purified materials are used, but almost immediately if a small quantity of alkali be added.

The phenomenon was first observed by Dubrunfaut [1] and ascribed by him to a change in molecular structure. He introduced the term bi-rotation because the rotatory power of glucose in solution is about twice as great when it is freshly dissolved as the value which it eventually assumes. With other sugars there exists no such exact numerical relation, and so the phenomenon was characterised as multi-rotation or pauci-rotation.

The general term mutarotation was introduced by Lowry [2] to describe the change in rotatory power, irrespective of its sign or magnitude, which takes place in a freshly prepared solution on standing.

In the sugar series the change is known to be due to the readiness in which the relative spatial positions of the groups attached to carbon 1 can be inverted. The interconversion of $\alpha$- and $\beta$-glucose is a mutarotation.

The process is in reality a special case of racemisation, which is limited to one of the several asymmetric carbons in the sugar molecule. The sugars remain, therefore, optically active after mutarotation, and the components in the equilibrium are in unequal proportions because the isomerides are unequally stable in the field of force created by the other asymmetric groups.

Generally speaking the sugar group shows great stability to racemisation; it is impossible to convert *d*-glucose to *l*-glucose except by an extremely roundabout method, and only in rare instances can racemisation at a given carbon atom be achieved.

Examples of racemisation of carbon 2 are in the Lobry de Bruyn interconversion of glucose and mannose by means of alkali, and the epimerisation of sugar acids.

In certain substitution reactions a Walden inversion may occur, with complete reversal of configuration at a particular carbon atom.

Emil Fischer [3] at first attributed mutarotation to a reversible hydration of the sugar. He noticed that the optical rotatory power of the sugar lactones altered as the lactone was hydrolysed to the corresponding acid and ascribed the change with glucose to a like addition of a water molecule forming glucose hydrate.

$$C_6H_{12}O_6 + H_2O \rightleftharpoons C_6H_{14}O_7.$$

The subject assumed a new aspect when it was shown by Tanret [4] in 1896 that two isomeric crystalline anhydrous forms of glucose could be obtained. α-glucose $[\alpha]_D + 110°$ on dissolving in water mutarotates, the rotatory power falling to $[\alpha]_D + 52{\cdot}5°$; similarly, β-glucose $[\alpha]_D + 19°$ on dissolving shows an increase of rotation to $[\alpha]_D + 52{\cdot}5°$. E. F. Armstrong was able to prove conclusively the direct relationship of α- and β-glucose to the α- and β-methylglucosides, for which a cyclic structure had been adopted. Simon [5] had come to a similar conclusion from the parallelism of optical rotatory power; molecular volume and molecular refractivity also confirm this conclusion.

| | $[\alpha]_D$. | $V_M$. | $R_G$. |
|---|---|---|---|
| α-Methylglucoside . . | + 158·9° | 132·61 ml. | 70·17 |
| α-Glucose . . . . | + 110·1° | 111·23 ml. | 62·68 |
| Difference | + 48·8° | + 21·38 ml. | + 7·49 |
| β-Methylglucoside . . | − 34·2° | 133·26 ml. | 70·55 |
| β-Glucose . . . . | + 19·3° | 111·65 ml. | 63·07 |
| Difference | − 53·5° | + 21·61 ml. | + 7·48 |

The idea became generally accepted that mutarotation was a reversible isomeric change α $\rightleftarrows$ β-glucose, exactly analogous to the mutarotation of nitrocamphor investigated by Lowry. It is a reversible conversion of isomeric forms of different rotatory power, not a complete conversion into another form as earlier workers had supposed.

Much interest has centred on both the mechanism of the interconversion α- $\rightleftarrows$ β-glucose, and the composition of the equilibrium mixture.

Lowry [6] for some time upheld the view that the open-chain aldehyde form of glucose or its hydrate was an intermediate in the

transformation. E. F. Armstrong formulated the change as due to formation and decomposition of an oxonium hydrate, the cyclic structure persisting unbroken.

Lowry has exhaustively investigated the mutarotation of glucose and glucose derivatives in a variety of solvents with a view to establishing the mechanism. It is found possible to arrest the mutarotation in certain solvents,[7] for example, tetramethyl glucose in chloroform and tetracetyl glucose in ethyl acetate. Mutarotation cannot take place spontaneously, but interaction with the medium is necessary. Striking is the arrest of mutarotation in dry pyridine or in dry cresol, a weak base and a weak acid respectively : a mixture of these two is, however, twenty times as effective a catalyst as water.

Mutarotation is effected by "acid basic catalysis" by the medium : for this there is necessary a solvent medium which is amphoteric, having both acid and basic properties. Water is a complete catalyst in this sense, as is a pyridine-cresol mixture, whereas either of these solvents alone is ineffective.

The catalysis is not an exclusive property of hydrogen or hydroxyl ions but is effected by any *acids* or *bases* in the sense used by Brönsted. The essential is a removal of a proton followed by its re-addition, during which process isomeric change may occur.

The speeds of mutarotation of a number of sugars are given in the following table, due to Hudson [8] :—

THE VELOCITY-COEFFICIENTS OF THE MUTAROTATION OF THE SUGARS IN WATER AT 20°.

| Sugar. | $k$. | Sugar. | $k$. |
|---|---|---|---|
| Fructose | 0·082 | α-Glucoheptose | 0·0122 |
| Lyxose | 0·065 | Galactose | 0·0102 |
| Rhamnose | 0·039 | Melibiose | 0·0088 |
| Arabinose | 0·031 | Maltose | 0·0072 |
| Fucose | 0·022 | Glucose | 0·0065 |
| Xylose | 0·021 | Cellobiose | 0·0047 |
| Mannose | 0·019 | Lactose | 0·0046 |

The mutarotation of glucose follows very closely the unimolecular law, and the velocity coefficients which have been determined over a range of temperature from 0° to 40° are identical for α- and β-forms. This identity also holds for other sugars. Mutarotation is thus a typical reversible isomeric change.

It is not logical to argue from these results that the equilibrated solution consists solely of α- and β-glucose and to calculate their proportions from the initial and final rotations as was attempted by Tanret. These calculated proportions are α-34 per cent., β-66 per cent., a ratio of 1 : 2 approximately.

It may be of interest, however, to give a table showing the proportion of α- and β-forms of a number of sugars which are present at equilibrium in aqueous solution as calculated from the rotations of the two forms and that at equilibrium, with the qualification that the simple equilibrium which is postulated is not the true state of affairs except as a first approximation.

Haworth and Hirst [9] point out that when the *cis* and *trans* relationships of the hydroxyl groups on the first and second carbon atoms are considered, it is found that the *trans* form invariably preponderates in the equilibrium mixture.

| Sugar. | *Trans* form. | Proportion of *trans* at equilibrium per cent. |
|---|---|---|
| *d*-Glucose | β | 66 |
| *d*-Mannose | α | 62 |
| *d*-Galactose | β | 69 |
| α-Glucoheptose | β | 89 |
| *d*-Xylose | β | 65 |
| *l*-Arabinose | α | 58 |
| Lyxose | α | 75 |
| Rhamnose | α | 73 |

The system $\alpha \rightleftarrows \mu \rightleftarrows \beta$ would, under certain conditions, also give unimolecular mutarotation curves whatever the proportion of the intermediate form. Furthermore, careful observations of rotation changes have shown that at 0° there are deviations from the unimolecular law with α- and β-glucose during the first few minutes of mutarotation. Riiber and Minsaas [10] observed a volume expansion in a freshly prepared α-glucose solution, followed by a contraction, and an initial absorption of heat followed a liberation of heat.

The changes during mutarotation are therefore complex.

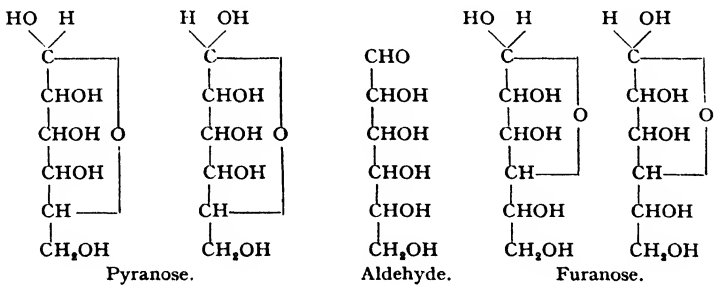
It is possible, however, to determine the proportion of α-glucose in the equilibrium, by measuring its solubility in a suitable solvent both before and after mutarotation has taken place. If time is allowed for mutarotation to occur, the total amount of sugar which passes into solution is naturally greater. This was done by Lowry.[11] Glucose is too soluble in water to make such measurements, but results were obtained with aqueous alcohol. In these experiments there was evidence that chemical changes were taking place which took longer to complete than the mutarotation as measured by rotation. The proportion of α-glucose was decreased by increasing the water concentration ; with 16 per cent. and 28 per cent. of water the concentration was 45 per cent. and 40 per cent. respectively. It could not be assumed that β-glucose was the only remaining constituent.

Hudson's [12] investigation of lactose showed that identical velocity constants were obtained whether the mutarotation was followed by rotation or by solubility measurements. This suggests that the mutarotation of lactose is a simpler process than that of glucose and of galactose. In the disaccharide lactose position 4 is substituted with a glucose residue, and therefore there is no hydroxyl group at this position free to take part in the formation of a $\gamma$-ring. These results strongly suggest that $\gamma$-sugars are present in the mutarotating solution of glucose. The chemical evidence that $\gamma$-glucose derivatives are obtained under various conditions from glucose solutions, also confirms this view. Chemical evidence cannot, and physical evidence does not yet give information as to the relative proportions of the different forms present.

It is the 5-ring $\gamma$-lactone of gluconic acid which is more stable than the 6-ring $\delta$-lactone. In the sugars, the stability is reversed, the 6-ring pyranose structure being more stable than the 5-ring furanose, but it cannot be assumed that the proportion of furanose sugar is negligible even in solutions from which only the $\alpha$- and $\beta$-pyranose sugars can be prepared by crystallisation.

The spectroscopic evidence referred to on page 26 indicates that even if there is some free aldehyde form of glucose in solution, its concentration is extremely small.

To conclude, the solution of glucose in water after mutarotation may, from the available evidence, contain at least five different molecular species, $\alpha$- and $\beta$-glucopyranose, $\alpha$- and $\beta$-glucofuranose, and the aldehyde.




Pyranose. Aldehyde. Furanose.

## Active Glucose.

Glucose may apparently exist in pyranose, furanose, aldehyde or enol forms, and the particular form in which it reacts is governed

by the physical conditions, but also by the nature of the reagent. Active glucose in this sense may be a different species with different reagents. For the purely chemical reactions of glucose there seems little reason to postulate an active glucose of special structure.

In biological systems, too, the conception of a specially active form seems an hypothesis of little practical value. It would seem sufficient to assume, for instance in fermentation, where the first stage is formation of hexose phosphate, that the enzyme merely serves to link the phosphoric acid with a form of the hexose already present, even if only in small amount, in the equilibrium. Whether the enzyme can also catalyse the re-establishment of the equilibrium is at present undetermined.

Riiber and others [13] have made very careful studies of the changes in volume and refractive index of solutions of sugars undergoing mutarotation. All the sugars which have been investigated, namely, glucose, fructose, galactose, mannose, arabinose and xylose, show departures from the unimolecular law. The simplest description of the results is obtained by fitting them to an empirical equation containing two exponential terms, corresponding to the system


More complex systems elude mathematical treatment.

Lowry and Smith [14] made a very detailed study of the mutarotation of $\alpha$- and $\beta$-galactose. Unlike glucose the unimolecular law is hardly approximated to, the velocity coefficient progressively falling. The results can be expressed by the equations for the simple system $\alpha \rightleftarrows \mu \rightleftarrows \beta$, and the relative proportions of the components at equilibrium were calculated as: $\alpha$- 28·5 per cent., $\beta$- 59·5 per cent., and $\mu$- 12·0 per cent. Riiber and Minsaas [15] made similar calculations from their measurements of specific volume and refractivity, and calculated the proportions as $\alpha$- 6·6 per cent., $\beta$- 66·0 per cent., $\mu$- 27·3 per cent. The considerable differences obtained by different methods show that the system is in fact more complex than that assumed, and show the limitations of the physical method for determining the number of species present.

We are not concerned here with the purely physicochemical studies of the phenomena of mutarotation for which reference should be made to the papers of Lowry who, with Smith, has given an excellent

and full historical summary in the report of the Dixième Conférence de l'Union Internationale de Chimie, Liége, 1930.

Mutarotation has been observed in a number of sugar derivatives which can be no more than listed here, viz. glucosamine hydrochloride, oximes, hydrazones, osazones, and anilides. Methylated and acetylated sugars in which the carbon 1 is unsubstituted show mutarotation, and their use by Lowry for studies of arrest of mutarotation has been indicated.

Mutarotation used in the wider sense to describe a reaction which can be followed polarimetrically is used to describe the reversible conversion of gluconic acid and its lactone, and of the analogous derivatives of other sugars, in aqueous solution.

The ketose sugars also show mutarotation, thus fructose $[\alpha]_D^{20}$ $-133{\cdot}5°$ changes to $[\alpha]_D^{20}$ $-92{\cdot}0°$; perseulose, turanose and lactulose also mutarotate. Tagatose, mannoketoheptose and glucoheptulose do not show mutarotation, and exist in α-forms as does sorbose, in which Hudson could detect no mutarotation. On the other hand, Riiber [18] finds that sorbose does show a feeble mutarotation.

The mutarotation of fructose [16] would appear to be a more far-reaching process than that of glucose: the equilibrium is not independent of the temperature, the rotation varying as follows:—

| T° | 0·15° | 15° | 25° | 37° |
|---|---|---|---|---|
| $[\alpha]_D$ | $-100°$ | $-94°$ | $-88°$ | $-81°$ |

Riiber and Esp [17] have followed the mutarotation of fructose at 20° by dilatometric, interferometric, calorimetric and polarimetric methods and obtained sensibly the same velocity constant by all the methods. The period of half change is $3\frac{1}{2}$ minutes. The volume increase is about six times that observed with glucose and the heat absorption about eight times.

If β-fructose is cautiously melted and then rapidly cooled, on dissolving in water the mixture contains initially more α-fructose than present in the equilibrium in water, since the rotation changes from $-63{\cdot}6°$ to $-92{\cdot}3°$, whereas β-fructose on dissolving in water changes from $-132{\cdot}5°$ to $-92{\cdot}3°$.

α-fructose has not been isolated in homogeneous condition

## The Relation of Rotatory Power to Configuration.

Most of the simpler carbohydrates exist in more than one form and show mutarotation. Before dealing with the quantitative relationships between rotatory power and configuration, a clear

statement is required on the nomenclature of the α- and β-derivatives of sugars of the *dextro* and *lævo* series. It was proposed by Hudson that in a *dextro* sugar the α-form of an α, β pair be defined as that which is most dextrorotatory, whereas in a *lævo* sugar the more lævorotatory (i.e. the less dextrorotatory) modification is regarded as the α-form.

On this basis α-*d*-glucose and α-*l*-glucose are optical antipodes. Ordinary crystalline maltose is thus β-maltose; crystalline fructose is β-fructose and not α-fructose as previously supposed.

The system is consistent and therefore useful, though it sacrifices the previous use of α and β to denote the same configuration for carbon 1 as that in α- and β-glucose.

The long known acetobromoglucose (*q.v.*) is called the α-form though it possesses the same configuration as β-glucose.

The principle of optical superposition first formulated by Van't Hoff stated that the optical rotation of a substance is additively composed of the algebraic sum of the contributions of the several asymmetric atoms: if the rotation due to any individual atom be $+\alpha°$, then on replacing that atom by its mirror image the contribution of the latter is $-\alpha°$.

Thus the four pentose sugars should have the following rotations contributed by the asymmetric centres:—

| No. 1. | No. 2. | No. 3. | No. 4. |
|---|---|---|---|
| $+a$ | $+a$ | $+a$ | $-a$ |
| $+b$ | $+b$ | $-b$ | $+b$ |
| $+c$ | $-c$ | $+c$ | $+c$ |

and since the sum of Nos. 2, 3 and 4 is equal to $+a+b+c$, the rotation of No. 1, which is probably the highest, should be equal to the rotation of the other three taken together. Given the rotations of the different pentoses, it is obviously possible to solve the equations to evaluate the contributions of each separate asymmetric carbon.

While there are series in which the Van't Hoff principle holds, it cannot be said to do so in general[18] as may be illustrated from the following four sugar acids:—

| | | | |
|---|---|---|---|
| COOH<br>\|<br>HCOH<br>\|<br>HOCH<br>\|<br>HCOH<br>\|<br>HCOH<br>\|<br>$CH_2OH$ | COOH<br>\|<br>HCOH<br>\|<br>HCOH<br>\|<br>HOCH<br>\|<br>HOCH<br>\|<br>$CH_2OH$ | COOH<br>\|<br>HOCH<br>\|<br>HCOH<br>\|<br>HCOH<br>\|<br>HOCH<br>\|<br>$CH_2OH$ | COOH<br>\|<br>HOCH<br>\|<br>HOCH<br>\|<br>HOCH<br>\|<br>HCOH<br>\|<br>$CH_2OH$ |
| *d*-Gluconic. | *l*-Mannonic. | *l*-Galactonic. | *d*-Talonic. |
| $\frac{M}{100}\cdot[\alpha]_D - 13°$ | $+1°$ | $+24°$ | $+33°$ |

These may be written in the form

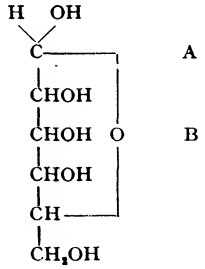
| | | | |
|---|---|---|---|
| $+a$ | $+a$ | $-a$ | $-a$ |
| $-b$ | $+b$ | $+b$ | $-b$ |
| $+c$ | $-c$ | $+c$ | $-c$ |
| $+d$ | $-d$ | $-d$ | $+d$ |

from which it is evident that if the Van't Hoff rule holds, the sum of the rotations of the four acids should equal zero. Actually the sum is $+45°$.

The optical superposition rule has in fact no theoretical foundation except as a first approximation.[18] Two groups which differ from each other in spacial sense alone may have an entirely different vicinal effect on the other groups attached to the same carbon atom. Stereoisomeric differences of the carbon atoms attached directly to the asymmetric carbon whose rotation is being considered invariably result in different vicinal effects on this carbon.

There are instances in the sugar group in which the superposition rule holds: in these the stereochemical differences are confined to carbons which are at least one removed from the asymmetric carbon which makes the major contribution to the rotation of the compound. Hudson has made such examples the basis of his rules of *isorotation*.

The contributions A of the potentially reducing group, and B of the rest of the sugar molecule are considered separately.




α-glucose $[M]_D = 20{,}300 = +A + B$,
β-glucose $[M]_D = 3{,}420 = -A + B$,

whence by subtraction $2A = 16{,}880$ and by addition $2B = 23{,}720$.

It was shown from the available data, firstly, that the difference between the molecular rotations of the α- and β-forms of the aldose sugars and their derivatives (2A) is a nearly constant quantity, and secondly, that the α- and β-forms of the derivatives of any aldose sugar have molecular rotations, the sum of which (2B) is equal to the sum for the α- and β-forms of the aldoses.

This method enables the calculation of the rotation of the unknown

isomerides of many of the sugars and their derivatives, and has proved of considerable value in elucidating their structure.

Hudson (summary paper in 10th Conférence de l'Union Internationale de Chimie, Liége, 1930) stated his rules of isorotation as—

1. The rotation of Carbon 1 is affected in only a minor degree by changes in the structure of the remainder of the molecule.
2. Changes in the structure of Carbon 1 affect in only a minor degree the rotation of the remainder of the molecule.

These two rules are illustrated by Hudson in the following tables:—

| Substance. | Observed in Water $[M]_D$. | Sum 2B. | Difference. 2A. |
|---|---|---|---|
| α-Methyl-*d*-glucoside | B + A = 30,830 | | |
| β-Methyl-*d*-glucoside | B − A = − 6,630 | | 37,460 |
| α-Methyl-*d*-xyloside | B′ + A′ = 25,200 | | |
| β-Methyl-*d*-xyloside | B′ − A′ = − 10,700 | | 35,900 |
| α-Methyl-*d*-gentiobioside | B″ + A″ = 23,318 | | |
| β-Methyl-*d*-gentiobioside | B″ − A″ = − 12,800 | | 36,118 |
| By the first rule | 2 A = 2 A′ = 2 A″ | | |
| α-Methyl-*d*-glucoside | B + A = 30,830 | | |
| β-Methyl-*d*-glucoside | B − A = − 6,630 | 24,200 | |
| α-*d*-Glucose | $B_1 + A_1$ = 20,300 | | |
| β-*d*-Glucose | $B_1 - A_1$ = 3,420 | 23,720 | |
| α-Glycol-*d*-glucoside | $B_2 + A_2$ = 30,350 | | |
| β-Glycol-*d*-glucoside | $B_2 - A_2$ = − 6,840 | 23,510 | |
| By the second rule | 2 B = 2 $B_1$ = 2 $B_2$ | | |

| Substance. | Observed in $CHCl_3$ $[M]_D$ | Difference. 2A |
|---|---|---|
| α-Methyl-*d*-xyloside triacetate | 34,700 | 52,300 |
| β-Methyl-*d*-xyloside triacetate | − 17,600 | |
| α-Methyl-*d*-glucoside tetracetate | 47,300 | 53,900 |
| β-Methyl-*d*-glucoside tetracetate | − 6,600 | |
| α-Methyl-*d*-galactoside tetracetate | 48,400 | 53,460 |
| β-Methyl-*d*-galactoside tetracetate | − 5,060 | |
| α-Methyl-gentiobioside heptacetate | 41,900 | 54,250 |
| β-Methyl-gentiobioside heptacetate | − 12,350 | |

It has been shown that the mutarotating sugars have as a common property a measurable maximum rate of solution, which is caused by the slow establishment in solution of the equilibrium between the α- and β-forms of the sugar; those sugars, such as sucrose, trehalose, raffinose, which do not reduce Fehling's solution or show mutarotation, do not exhibit this maximum rate of solution. Values for the rotatory powers of those sugars for which both modifications have not been crystallised and measured directly can be obtained by

measuring the maximum rate of solution, or the initial and final solubilities—e.g. for xylose, arabinose, lyxose, rhamnose, mannose, fructose, glucoheptose, maltose, cellobiose.

The results are summarised in the following table :—

ROTATORY POWERS OF THE MUTAROTATING SUGARS.

| Sugar. | M.W. | Formula. | Specific Rotation in Water. | | | Molecular Rotation Difference. 2A. |
|---|---|---|---|---|---|---|
| | | | α-Form. | Constant Rotation. | β-Form. | |
| *d*-Glucose | 180 | $C_6H_{12}O_6$ | + 113·4° | + 52·2° | + 19° | + 16,900 |
| *d*-Galactose | 180 | $C_6H_{12}O_6$ | + 144·0° | + 80·5° | + 52° | + 16,600 |
| *d*-Mannose | 180 | $C_6H_{12}O_6$ | *+ 34°* | + 14·6° | − 17° | + 9,180 |
| *d*-Xylose | 150 | $C_5H_{10}O_5$ | + 92° | + 19° | *− 20°* | + 16,800 |
| *d*-Lyxose | 150 | $C_5H_{10}O_5$ | + 5·5° | − 14° | *− 36°* | + 6,220 |
| *d*-Arabinose | 150 | $C_5H_{10}O_5$ | *− 54°* | − 105° | − 175° | + 18,100 |
| *l*-Rhamnose * | 164 | $C_6H_{12}O_5$ | − 7·7° | + 8·9° | *+ 54°* | − 10,000 |
| α-Glucoheptose | 210 | $C_7H_{14}O_7$ | *+ 45°* | − 20·4° | − 28·4° | + 15,300 |
| Lactose | 342 | $C_{12}H_{22}O_{11}$ | + 90·0° | + 55·3° | + 35° | + 18,800 |
| Maltose | 342 | $C_{12}H_{22}O_{11}$ | *+ 168°* | + 136° | + 118° | + 17,100 |
| Melibiose | 342 | $C_{12}H_{22}O_{11}$ | *+ 179°* | + 142·5° | + 124° | + 18,800 |
| Cellobiose | 342 | $C_{12}H_{22}O_{11}$ | *+ 72°* | + 35° | + 16° | + 19,200 |

Calculated values in italics.

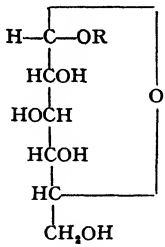
* The negative sign of 2A for rhamnose is due to this being a *l*-sugar.

The relative ease with which the isomeric fully acetylated derivatives are prepared and purified makes them especially suitable for investigating the relation of rotation and constitution. The difference between the molecular rotations of the α- and β-sugars should be a constant, and the following figures show this to be fairly true :—

| Substance. | Molecular Rotation of α-form. | Molecular Rotation of β-form. | Difference. |
|---|---|---|---|
| *d*-Glucose pentacetate | + 39,600 | + 1,500 | + 38,100 |
| *d*-Lactose octacetate | + 36,500 | − 2,900 | + 39,400 |
| *d*-Maltose octacetate | + 83,000 | + 42,500 | + 40,500 |
| *d*-Cellobiose octacetate | + 27,800 | − 10,200 | + 38,000 |
| *d*-Glucosamine pentacetate | + 36,400 | + 470 | + 35,930 |
| *d*-Chondrosamine pentacetate | + 39,500 | + 4,100 | + 35,400 |
| *d*-Gentiobiose octacetate | + 35,500 | − 3,600 | + 39,100 |
| *d*-α-Glucoheptose hexacetate | + 40,200 | + 2,200 | + 38,000 |
| *d*-Mannose pentacetate | + 21,400 | − 9,800 | + 31,200 |
| *d*-Galactose pentacetate | + 41,600 | + 8,900 | + 32,700 |
| *d*-Xylose tetracetate | + 28,300 | − 7,900 | + 36,200 |
| *l*-Arabinose tetracetate | + 13,400 | + 46,800 | − 33,400 |

While some sugars and their derivatives obey the isorotation rules, other sugars show great irregularities. The deviations are best

discussed after considering in detail the rotations of the four hexoses, glucose, galactose, gulose and mannose and of their methylglycosides.[19]



R = H

| Glucose. | Galactose. | Gulose. | Mannose. |
|---|---|---|---|
| α + 203° | + 259° | + 113° | + 54° |
| β + 34° | + 94° | unknown | − 31° |
| 2A 169° | 165° | — | 85° |

R = Me

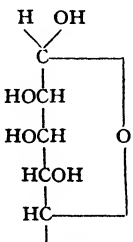
| Methyl-glucoside. | Methyl-galactoside. | Methyl-guloside. | Methyl-mannoside. |
|---|---|---|---|
| α + 308° | + 374° | + 206° | + 153° |
| β − 66° | − 1° | − 161° | − 132° |
| 2A 374° | 375° | 367° | 285° |

The above rotations are expressed as $[M]_D/100$.

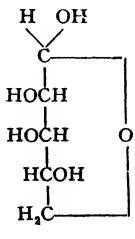
It is evident that while the first three sugars conform to the first isorotation rule, mannose does not, and that the rotation A of $C_1$ is affected by stereochemical changes in the rest of the molecule.

It is to be noticed that glucose, galactose and gulose have the same configuration for $C_2$, while mannose and the two sugars lyxose and rhamnose, which also fail to obey the isorotation rule, possess the epimeric structure.

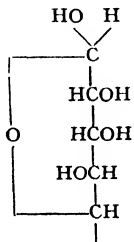
Hudson [20] was led to explain the failure to obey the isorotation rule by the hypothesis that sugars of the configuration possessed in common by rhamnose, lyxose or mannose



*d*-Mannose.



*d*-Lyxose.



*l*-Rhamnose.

did not possess a normal ring structure and were in fact furanose sugars, and therefore did not lend themselves to numerical comparisons with the other pyranose structures. Such an idea is, however, in conflict with the structure determined by methylation, which has shown these sugars to be normally constituted, and with their known chemical properties.

It is more logical therefore to explain the failure of the isorotation rule here, by the fact that it is inapplicable since the Van't Hoff rule of optical superposition on which it is based is itself an approximation.

Haworth [21] has found that in the mannose series the deviations in rotation depend to a large extent on the solvent in which they are measured, and that it is dangerous to draw conclusions as to ring structure from measurements in one solvent only.

In glucose, galactose and gulose the principle of superposition is applicable because the stereochemical differences are removed by one atom from $C_1$ and do not operate on it; in the mannose series $C_2$ is inverted and the difference in the vicinal effect affects the rotation of $C_1$.

The applicability of the second isorotation rule may be tested by comparing 2B for a number of glucosides.

| | $2B : [M]_D/100$ |
|---|---|
| Glucose | 237 |
| Methylglucoside | 242 |
| Ethylglucoside | 252 |
| Glycolglucoside | 236 |
| Phenylglucoside | 277 |

It is evident that the radicals OH, $OCH_3$, $OC_2H_5$, and $OCH_2 . CH_2OH$ have a similar effect on the rest of the molecule, but that the phenyl radical behaves differently. A limitation of rule 2 is revealed.

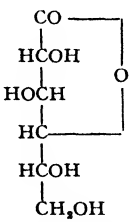
It is possible to calculate the contributions of the individual asymmetric carbons to the optical rotation of the sugar molecule as a whole by solving the necessary simultaneous equations. Owing to the limitations of the working hypothesis, the figures obtained starting with different sets of equations are not necessarily the same and sometimes contradictory. The results are, however, of interest, and a series of values calculated by Isbell [22] are given.

The table shows the sign and magnitude of the molecular rotation for the contribution of each H—C(|)—OH group (R in the table).

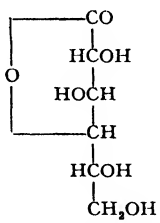
| Hexose Series. | | Pentose Series. | |
|---|---|---|---|
| Sugars. | Methylglycosides. | Sugars. | Methylglycosides. |
| $\alpha OH = +8{,}350$ | $\alpha Me = +18{,}250$ | $\alpha OH = (+8{,}350)$ | $\alpha Me = +18{,}375$ |
| $R_2 = +7{,}450$ | $R'_2 = +7{,}650$ | $r_2 = +6{,}490$ | $r'_2 = +7{,}750$ |
| $R_3 = -7{,}400$ | $R'_3 = -8{,}200$ | $r_3 = -5{,}185$ | $r'_3 = -6{,}290$ |
| $R_4 = -2{,}875$ | $R'_4 = -3{,}240$ | $r_4 = -6{,}225$ | $r'_4 = -7{,}150$ |
| $R_5 = -100$ | $R'_5 = -400$ | | |

The rough general agreement among these figures justifies the assumption that the sugars and their methylglycosides have similar structures. Apparently the replacement of hydroxyl by methoxyl on $C_1$ affects somewhat the rotation of all the carbon atoms, a concept not in agreement with the rigid application of Hudson's second rule of isorotation.

The sign and magnitude of the optical rotatory power of certain derivatives enable a considerable insight to be gained into the configuration of the molecule. For example, it is established [23] in twenty-four lactones of the monobasic sugar acids and eleven lactones of the saccharinic acids that polarised light is rotated to the right or to the left according as the $\gamma$-lactone ring on $C_4$ is on the right or left of the projection formula. Accordingly, the configuration of the fourth carbon atom of any new lactone can at once be determined from its rotation. This is illustrated by the lactones of gluconic and galactonic acids :—

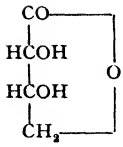



Gluconolactone
$[\alpha]_D + 68°$.

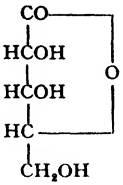



Galactonolactone
$[\alpha]_D - 77{\cdot}0°$.

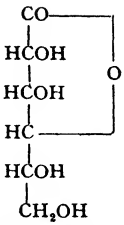
Certain exceptions to the lactone rule are known. Thus the following acids have the OH on $C_4$ to the right but give lævorotatory $\gamma$-lactones, *d*-erythronic, digitoxonic, *d*-allonic and *d*-mannonononic, while *d*-ribonic acid is but weakly dextrorotatory.



*d*-Erythronolactone.
$[\alpha]_D - 73°$.



*d*-Ribonolactone.
$[\alpha]_D + 14°$.



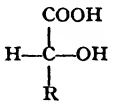
*d*-Allonolactone.
$[\alpha]_D - 6°$.

A comparison of these formulæ and a knowledge of the contributions of each asymmetric centre H—C—OH enables an extended lactone rule, covering the exceptions, to be put forward (Freudenberg).[18] The contributions for each asymmetric carbon are 2, — 10° ; 3, — 43° ; 4, — 86° ; 5, — 55° in the hexonic lactones. It is evident why $C_4$ dominates in deciding the sign of the rotation, and also apparent how in the quoted exceptions the other groups could, combined, confer a lævorotation.

In its generalised form the lactone rule states that a $\gamma$-lactone will be dextrorotatory when $C_4$ has the configuration H—C—OH with the hydroxyl group to the right, and when at least one of the other groups has the reverse configuration. If all the groups have the same configuration as $C_4$ the lactone will be feebly dextrorotatory or lævorotatory.

By adding one group through the cyanhydrin synthesis, or by degradation of an aldose to a lower sugar, it is possible by use of the lactone rule to obtain the configuration of each asymmetric carbon of an aldose : Hudson investigated the structure of rhamnose and gluco-octose, and Clark [24] that of fucose in this manner.

The sign of rotation of the amides of all $\alpha$-hydroxyacids is determined by the configuration of $C_2$ independent of R in the formula : when its hydroxyl is on the right the amide is dextrorotatory and conversely.



The rule is very useful for determining the configuration of $C_2$ in aldonic acids. There are a few exceptions where the contribution

of R causes a lævorotation and Freudenberg adapts the rule to include these, in the generalisation that when an amide is more dextrorotatory than the free α-hydroxy-acid, the latter has the above configuration.

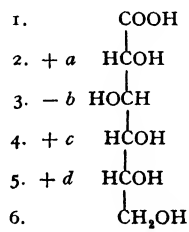
The rotations of a sufficient number of amides are known to permit the calculation of the molecular rotations of the individual asymmetric carbon atoms.[25]

| Carbon Atom. | Pentonic Amides. | Hexonic Amides. | Heptonic Amides. |
|---|---|---|---|
| 2 | + 4450 | + 4725 | + 4585 |
| 3 | − 2315 | − 1465 | − 1960 |
| 4 | + 575 | + 95 | ? |
| 5 | | − 205 | − 420 |

The rotation of carbon 2 which is by far the largest is little influenced by the change in the length of the chain.

Similar deductions as to the configuration of $C_2$ may be made from the direction of rotation of the phenylhydrazide of the acid.[26] If the phenylhydrazide rotates to the right the hydroxyl on $C_2$ is on the right, and vice versa.

In gluconic acid, there are four asymmetric carbon atoms, 2, 3, 4, 5, and the molecular rotation may be expressed as $+ a$, $- b$, $+ c$, $+ d$, the + sign indicating an hydroxyl on the right.




Hudson [27] has deduced values for $a$, $b$, $c$ and $d$ by solving the four equations for the phenylhydrazides of gluconic, gulonic, idonic and galactonic acids, for which the rotations have been measured by Nef. He finds the comparative values ($\times 10^2$) are

$$a + 37{\cdot}3^\circ, \quad b + 3{\cdot}9^\circ, \quad a + 1{\cdot}4^\circ, \quad c - 0{\cdot}6^\circ,$$

showing that the value of the rotation of $C_2$ is so very much larger than the values of the rotations of the other three carbons that its sign and configuration determines the sign of the rotation of the whole molecule.

Levene [26] finds for the salts of the monobasic acids that the con-

figuration of the α-carbon atom has a strong influence on the rotation. The values calculated from his observations are

$$a + 22°, \quad b + 13°, \quad c + 12°, \quad d - 4°.$$

These are quite different from those found for phenylhydrazides and amides, and it is evident further that the contribution of $C_2$ is less than that of the sum of the other three carbons, and therefore less decisive for determining configuration.

Further data are provided by Marle [28] and by Wijk.[29]

The former has shown that Hudson's law holds equally for the hydrazides, *p*-bromophenylhydrazides, *o*-, *m*- and *p*-tolylhydrazides, amides, anilides, and toluides of the monobasic acids, the rule being applicable for aqueous solutions but not for solutions in pyridine. The latter has applied it to methylamides, benzylamides and β-phenylethylamides. Both papers contain useful data on the effect exercised by various substituents on the rotation.

The benzylphenylhydrazones of the sugars rotate to the left when the asymmetric α-carbon atom has its hydroxyl to the right, and vice versa. Votoček [30] concludes that the benzylhydrazone residue so exalts the effect of the α-carbon that its hydroxyl determines the sign of rotation of the whole molecule.

## REFERENCES TO CHAPTER IV.—MUTAROTATION.


1. Dubrunfaut, Compt. rend., 1846, **23**, 38.
2. Lowry, J.C.S., 1899, 211.
3. Fischer, Ber., 1890, **23**, 2626.
4. Tanret, Compt. rend., 1895, **120**, 1060.
5. Simon, Compt. rend., 1901, **132**, 487.
6. Lowry, J.C.S., 1903, 1314.
7. Lowry and Richards, J.C.S., 1925, 1385. Lowry and Owen, Proc. Roy. Soc., 1928, **119**, 505.
8. Hudson, J.A.C.S., 1910, 889.
9. Haworth and Hirst, J.C.S., 1928, 1221.
10. Riiber and Minsaas, Ber., 1926, **59**, 2266.
11. Lowry, J.C.S., 1904, 1551.
12. Hudson, J.A.C.S., 1904, 1065.
13. Riiber, Tidsskr. Kjemi. Berg., 1932, **10**, 252.
14. Smith and Lowry, J.C.S., 1928, 666.
15. Riiber, Minsaas and Lyche, J.C.S., 1929, 2173.
16. Nelson and Beegle, J.A.C.S., 1919, 559.
17. Riiber and Esp, Ber., 1925, **58**, 737.

THE RELATION OF ROTATORY POWER TO CONFIGURATION.

18. Freudenberg and Kuhn, Ber., 1931, **64**, 703.
19. Kuhn and Freudenberg, Drehung der Polarisationsebene des Lichtes, p. 31; Leipzig, 1932.
20. Hudson, J.A.C.S., 1930, 1680; 1707.
21. Haworth, Hirst and Smith, J.C.S., 1930, 2659.
22. Isbell, Bur. Stan. J. Res., 1929, **3**, 1041.
23. Hudson, J.A.C.S., 1910, 338; J.A.C.S., 1917, 462. Anderson, J.A.C.S., 1912, 51.
24. Clark, J.B.C., 1922, **54**, 65.
25. Hudson, J.A.C.S., 1918, 813; J.A.C.S., 1919, 1141.
26. Levene, J.B.C., 1915, **23**, 145; J.B.C., 1917, **31**, 623.
27. Hudson, J.A.C.S., 1916, 462.
28. Marle, Rec. trav. chim., 1920, **39**, 540.
29. Wijk, Rec. trav. chim., 1921, **40**, 221.
30. Votoček, Valentin, Leaminger, Coll. Czech. Chem. Comm., 1931, **3**, 250.

## CHAPTER V.

### THE CHEMICAL PROPERTIES OF GLUCOSE AND THE HEXOSES.

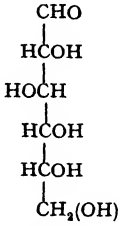
GLUCOSE, the other aldoses and the ketoses are easily oxidised; this is evidenced by their activity as reducing agents. They reduce alkaline copper solutions on warming, forming red cuprous oxide, likewise ammoniacal silver solutions forming a metallic mirror. When heated with alkali, a sugar solution colours at first yellow, subsequently brown as it decomposes; a variety of substances, including lactic acid and other hydroxy acids, are formed. Valuable analytical methods for the estimation of glucose are based on the reaction with copper salts in alkaline solution, but the precise changes which the sugar undergoes under these conditions are incompletely understood.

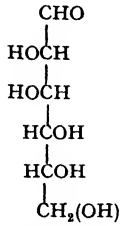
The sugars behave as very weak polybasic acids. The most acid hydroxyl groups have a dissociation constant of about $1{\cdot}0 \times 10^{-12}$ and the other hydroxyl groups dissociate at high alkali concentrations. The strongly alkaline solutions of alkali metal glucosates undergo far-reaching decomposition, but the alkaline earth metals yield insoluble compounds with the sugars.

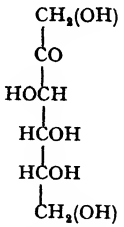
### Interconversion of Glucose, Fructose and Mannose.

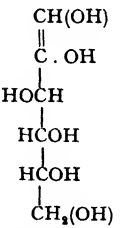
Glucose, fructose and mannose are interconvertible in aqueous solution in presence of alkali. This most important transformation was first observed by Lobry de Bruyn and Van Ekenstein;[1] it takes place slowly at ordinary temperatures, quickly and with much secondary decomposition at higher temperatures. The interconversion of glyceraldehyde and dihydroxyacetone in aqueous solution in presence of bases is exactly analogous. Starting with glucose or with mannose the optical rotation falls, and with fructose it increases to a constant value. The final mixture contains all three sugars but not necessarily in the same proportions starting with any one of the three different hexoses. Wohl[2] explained the interconversion as taking place through an enolic form common to all three sugars. It is not known whether the enol is formed directly from the ring form

of the sugar or through the aldehyde. The enol can revert to any one of the three sugars.


Glucose.

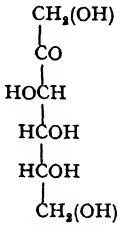

Mannose.

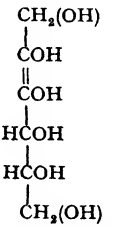

Fructose.

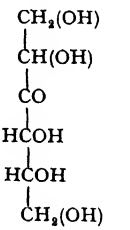

Enolic form.

Whilst the interconversion of glucose, mannose and fructose is a reversible process, no true equilibrium is obtained owing to the secondary irreversible changes which occur.

Fructose can give rise to a second enolic form, which can revert either to fructose or to a new ketose known as glutose.


Fructose.


Second Enolic form.

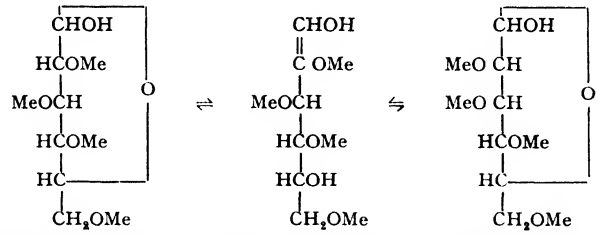

Glutose.

Lobry de Bruyn isolated glutose as a regular product of the transformation of glucose, finding it to the extent of 4·8 per cent. in Louisiana molasses : mannose has also been found in molasses. Neither glutose nor mannose is a product of the sugar cane, but arise by the action of the lime used in refining.

The interconversion can be effected with weak alkalis, with weak bases such as guanidine, and even with neutral sodium phosphate solution. Wolfrom and Lewis [3] found that in saturated calcium hydroxide solution glucose was converted into a mixture of 63·4 per cent. glucose, 30·9 per cent. fructose and 2·4 per cent. mannose together with 3·3 per cent. non-sugar substances, probably saccharinic acids.

In glucose solutions no proof of the intervention of an enol form can be had, but Wolfrom and Lewis found evidence of an intermediate enol in the interconversion of tetramethyl-glucose and tetramethyl-mannose. No ketose can form here, and a simple equilibrium

consisting of equal parts of the two tetramethyl-hexoses is obtained; the equality is fortuitous.




Tetramethyl-glucose. Common Enolic form. Tetramethyl-mannose.

It is suggested that since only the 1 : 2 enol can form, the interconversion is due to migration of hydrogen from $C_2$ to $C_1$ and not to hydration followed by dehydration.

The equilibrated mixture showed a higher iodine absorption than could be accounted for by a simple mixture of the tetramethyl aldoses: this was attributed to the presence of an unsaturated enol, since after acidifying the iodine absorption fell to normal, owing to reconversion of the enol to the normal sugars.

The following table gives the composition of the final mixture obtained by Spoehr and Strain [4] starting with each of the three hexoses in disodium phosphate of $p_H$ 8·2 :—

| Initial Sugar. | Percentages of Products in Equilibrium. | | | |
|---|---|---|---|---|
| | Aldoses. | Ketoses. | Mannose. | Glucose. |
| *d*-Glucose . . | 78 | 22 | 2 | 76 |
| *d*-Mannose . . | 68 | 32 | 46 | 22 |
| *d*-Fructose . . | 39 | 71 | 10 | 29 |

There was a definite small production of saccharinic acids, and it is uncertain whether the conversion stopped because equilibrium was being approached or because the acidity increased. Fructose in solutions of acid phosphate of $p_H$ 6·6 shows an increase in rotation and a conversion to other hexoses. There was no evidence of the formation of hexose phosphates.

The properties of glutose have been studied by Benedict, Dakin and West.[5] It is unfermentable, and does not form a hexose phosphate and is not utilised by the diabetic organism. It is excreted unchanged in greater part. Spoehr and Wilbur [6] found that glutose could be prepared from glucose or invert sugar in disodium phosphate solution

at 70°. Fermentable sugars are removed with starch-free yeast and a 50 per cent. yield of glutose can be obtained. It is not possible to reverse the process and obtain glucose or fructose by the action of alkalis on glutose. There is considerable doubt whether glutose has the structure which is assigned to it, and there is evidence that the syrup which is called glutose is by no means a homogeneous product.

*d*-Galactose yields with alkalis a mixture containing galactose, talose, tagatose, sorbose and galtose. The changes only involve carbon 1, 2, and 3, and a conversion to any member of the glucose series, which would involve an inversion of $C_4$, has never been observed

The Lobry de Bruyn conversion has proved useful for preparing new sugars. Thus Montgomery and Hudson [7] prepared the related ketose disaccharide, lactulose from lactose, and Austin [8] made glucoheptulose from glucoheptose.

During the sterilisation of media containing sugars, to be used as culture media for bacteria, etc., extensive degradation of glucose is brought about,[9] a fact which should be borne in mind when interpreting the results. For this reason the inorganic constituents and the glucose are often separately sterilised and then mixed. It appears that twenty minutes at 115° is a better practice than three discontinuous thirty-minute treatments at 100°. In the former there is 28 per cent. loss of reducing power and in the latter 40 per cent. The acidity increases during sterilisation.

## Action of Stronger Alkalis.

In stronger alkalis sugars undergo more drastic degradation. In the absence of oxidising agents saccharinic acids are formed, one part of the sugar molecule being reduced and another simultaneously oxidised.

Twenty-four isomeric acids with six carbon atoms are theoretically possible, viz. :—

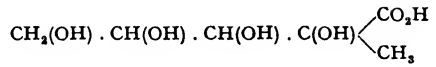
(1) Eight stereoisomeric metasaccharinic acids,

$$CH_2(OH) . CH(OH) . CH(OH) . CH_2 . CH(OH) . CO_2H$$

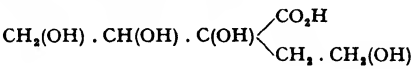
(2) Four isosaccharinic acids,

$$CH_2(OH) . CH(OH) . CH_2 . C(OH)\begin{matrix}CO_2H\\CH_2OH\end{matrix}$$

(3) Eight saccharinic acids,



(4) Four parasaccharinic acids,



A number of the acids have been prepared and studied, princi pally by Kiliani [10] and by Nef.[11] A discussion of Nef's theories on the mechanism of their formation is outside the scope of this monograph.

The formation by purely chemical means of three carbon bodies by alkaline degradation of glucose is important because of the parallel with the breakdown of the sugars in fermentation, in muscle contraction and biological processes generally, to similar compounds.

Fischler [12] found that by the action of sodium bicarbonate in presence of sodium sulphite on glucose solutions, glyceraldehyde or dihydroxy acetone, isolated as their osazone, was produced.

Similarly, weak alkalis act on most sugars on heating the solution to yield methyl glyoxal $CH_3CO \cdot CHO$, which, in presence of phenylhydrazine, can be isolated as its osazone. Distillation of alkaline solutions of sugar also yields methyl glyoxal.

A very elaborate study of the action of alkalis on carbohydrates extending over ten years was made by Nef.[13] As a result, a relatively consistent explanation can now be given of the behaviour of any carbohydrate in aqueous alkali hydroxides towards oxidising agents such as air, hydrogen peroxide or the oxides of mercury, silver and copper. In the course of this work a number of the sugar acids and their lactones, salts, etc., have been fully characterised.

According to Nef, any carbohydrate in weak alkaline solution undergoes profound change, and is eventually transformed into an equilibrated mixture in which no less than one hundred and sixteen substances can in theory take part. These are the thirty-two aldoses with one to six carbon atoms, the thirty-two corresponding methylenols, the twenty-six ketoses with three to six carbon atoms in an unbranched chain and the twenty-six dienols. Actually in practice only ninety-three different substances are found.

In the absence of an oxidising agent the different sugars are converted into saccharinic acids, while in the presence of air or other oxidising agents the oxidation of the sugars results in the formation of carbon dioxide, formic, glycollic, oxalic and *dl*-glyceric acids, four trihydroxy butyric acids, eight tetrahydroxy valeric acids, and eight

tetrahydroxyhexoic acids, all of which have been isolated and identified.

The degradation of the sugar molecule is represented as taking place through fission at the double bond of the various dienols : thus an aldohexose will furnish the following products :—

1 : 2 dienol → formaldehyde + pentose.
2 : 3 dienol → glycollaldehyde + tetrose.
3 : 4 dienol → glyceraldehyde.

Some of these products may condense together again under the influence of the alkali : others may themselves undergo the Lobryn de Bruyn rearrangement yielding new products : oxidising agents may, on the other hand, oxidise the dienols directly giving two molecules with acid groups.

This mechanism enables all the known products of alkaline oxidation to be predicted.

Nef found among the products from glucose :—

By direct oxidation → gluconic and mannonic acids.

1 : 2 dienol → *d*-arabonic and *d*-ribonic acid + formic acid.
2 : 3 dienol → *d*-erythronic acid + *l*-threonic acid + glycollic acid.
3 : 4 dienol → glyceric acid.

The relative stabilities of intermediates are different for different sugars, and very different proportions of the different types of product are therefore obtained. Oxidation is also accompanied by the production of some resinous products.

This explains why the isomeric sugars do not give the same results when titrated with Fehling's solution. Nef found the following relative figures :—

*d*-glucose 100 ; *d*-mannose 102 ; *d*-fructose 93·4 ; *d*-galactose 92·5 ; *d*-tagatose 81·2 ; *l*-sorbose 82·1.

It is to be expected that the methylated sugars, owing to protection of the hydroxyl groups, will be more stable to alkaline oxidation than the sugars themselves. In agreement with this, it is found that all methylated glucoses have less reducing power,[14] as measured by the method of Bertrand, than glucose itself. 2 : 3-dimethylglucose has less reducing power than 3 : 4 : 6-trimethylglucose, which is due to the fact that the free hydroxyl group on $C_2$ in the latter substance permits enolisation. The reducing power of pentamethylaldehydoglucose towards alkaline copper solutions approximates to its reducing power towards hypoiodite ; the latter, being a measure of the reducing power of the aldehydic group only,[15] is equal for glucose itself and all its methyl ethers.

## Action of Ammonia.

Glucose can be made to yield an aldehyde ammonia addition product, according to Ling and Nanji,[16] by passing dry ammonia into a suspension of glucose in methyl alcohol,

$$CH_2OH(CHOH)_4\,CH(OH)NH_2.$$

It melts 123°-124° with decomposition, and has $[\alpha]_D$ + 20·3°. It reduces Fehling's solution and ammoniacal silver nitrate, and would appear to be a typical aldehyde-ammonia. It is completely dissociated in aqueous solution.

The action of strong ammonia is more interesting, as the ammonia combines with the products of alkaline breakdown. Windaus and Knoop [17] found that in strong ammonia solution, saturated with zinc hydroxide to increase the alkalinity, methyl glyoxaline is formed,

$$CH_3 . CO . CHO + 2NH_3 + HCHO \rightarrow \begin{matrix} CH_3 . C . NH \\ \| \quad\quad \diagdown \\ CH . N \quad\quad CH \end{matrix}$$

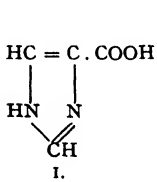
This is explained by a dissociation of the sugar to yield glyceraldehyde which changes to methyl glyoxal which reacts with formaldehyde, also a dissociation product, and with ammonia. In presence of acetaldehyde dimethyl glyoxaline is produced.

Windaus [18] found that the reaction is not confined to glucose, but that the same methyl glyoxaline is yielded by mannose, fructose, sorbose, arabinose, xylose and rhamnose, or by the disaccharide lactose.

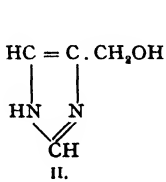
The formation of an imidazole ring in this way is of considerable interest in view of its common occurrence in plant and animal products. It occurs in histidine, in ergothionine, in alkaloids such as pilocarpine, and in the purines.

Windaus and Ullrich [19] found imidazole 4-carboxylic acid (I) to be a product of the action of ammonia solutions containing copper hydroxide on glucose. This method for the production of imidazole derivatives from sugars has lately been studied by Parrod.[20]

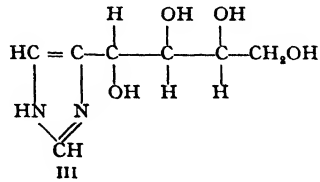
Fructose gives in ammoniacal copper hydroxide, through which air is passed, 4-oxymethyl imidazole (II.), and also *d*-arabinotetraoxybutyl-4-imidazole (III.), which is also given by mannose and glucose and whose constitution is proved by its synthesis from glucosone, ammonia and formaldehyde.



I.



II.



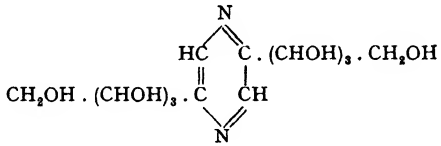
III

Galactose yields the corresponding *d*-lyxo-tetraoxybutyl-4-imidazole.

Fructose yields hydroxymethyl imidazole even under the mild conditions of a molar solution containing ammonium and copper carbonates at $p_H$ 7·7 on passing in air. In ammoniacal solution using methylene blue as oxygen carrier fructose yields the amide of imidazole-4-carboxylic acid.

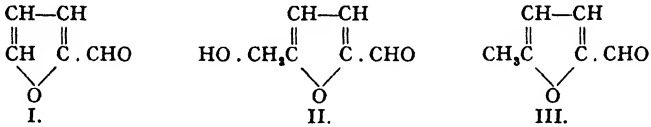
Another example of a derivative formed by the action of ammonia, in which part of the sugar skeleton remains unaltered, is obtained by the action of methyl alcoholic ammonia on fructose, and also from glucosamine solutions which are left to stand in the air to condense and oxidise.[21]

Stolte [22] has shown the product to be 2 : 5-ditetrahydroxybutylpyrazine.



## Action of Acids.

The sugars themselves are in the cold stable towards acids, an acid solution having the same value for the rotation as an aqueous solution. On heating, however, they break down. Heating with 12 per cent. hydrochloric acid results in the formation of furfural I. from pentoses; hydroxymethylfurfural II. and lævulinic acid; $CH_3 . CO . CH_2 . CH_2 . CO_2H$ from the hexoses; methylfurfural III. from the methylpentoses.



The production of furfural is quantitative, and one of the methods for the estimation of the pentoses [23] depends on the precipitation of the furfural as its insoluble phloroglucinol compound.

Furfural is made technically in large quantities from oat hulls by heating with sulphuric acid : a 10 per cent. yield is obtained from this source.

## Interaction with Phenyl Hydrazine.

Particularly characteristic is the behaviour of the sugars with excess of phenyl hydrazine on heating in dilute acetic acid solution. With glucose an orange-yellow insoluble phenylosazone is formed, which serves to characterise glucose even when present only in very small quantities, though not to distinguish it from some of the isomeric hexoses which give the same or closely related phenylosazones. The use of phenyl hydrazine possesses further a historical interest, as in the hands of Emil Fischer [24] it served as one of the chief aids in the elucidation of the chemistry of the carbohydrates.

Glucose and phenyl hydrazine interact in acid solution, acetic acid being usually employed, in two stages. In the first, which takes place in cold solution, a phenyl hydrazone is formed—

$$C_6H_{12}O_6 + C_6H_5 . NH . NH_2 = C_5H_{11}O_5 . CH : N . NH . C_6H_5 + H_2O$$

This is a colourless compound, soluble in water, existing in two modifications, one or other of which is obtained according to the method of preparation.

Skraup's [25] $\beta$-phenyl hydrazone, formed by shaking glucose with phenyl hydrazine in alcoholic solution, crystallises in needles, m.p. 106°-107°, and has an optical rotation in aqueous solution of $[\alpha]_D$ — 2° changing to — 50°. Fischer's $\alpha$-isomeride, formed in alcoholic acetic acid solution, crystallises in leaflets, m.p. 159°-160°, $[\alpha]_D$ — 70° changing to — 50°. Behrend [26] has shown Skraup's $\beta$-isomeride to be in reality a compound of phenyl hydrazine (1 mol.) with 2 molecules of the $\beta$-hydrazone. This hydrazone also forms an additive compound with pyridine which, on treatment with alcohol, yields glucose $\beta$-phenyl hydrazone, m.p. 140°-141°, $[\alpha]_D$ — 5·5°. Behrend has advanced evidence to show that this is a true hydrazone,

$$CH_2(OH) . [CH(OH)]_4 . CH : N . NHPh,$$

whereas Fischer's glucose $\alpha$-phenyl hydrazone is a hydrazide—

$$CH_2(OH) . CH . [CH(OH)]_3 . CH . NH . NHPh.$$

This view is upheld, in preference to the alternative possibility of regarding them as derivatives of $\alpha$- and $\beta$-glucose, by Frère-Jacque,[27] the evidence for this view being that on hydrolysis with oxalic acid both phenyl hydrazones yield a solution of glucose of which the rotation falls on making alkaline, and therefore contains $\alpha$-glucose.

Alkali hydroxide increases the rate of mutarotation of glucose, but greatly decreases the rate of mutarotation of the phenyl hydrazones.

The phenyl hydrazones of glucose and most of the other sugars, being easily soluble, are not adapted for characterising the parent sugars. An exception is afforded by mannose, which forms an almost insoluble phenyl hydrazone and can thus be very readily detected. This compound affords an illustration of the influence exercised by small differences in configuration of the molecule on its physical properties. Sparingly soluble phenyl hydrazones are also formed by the methyl pentoses.

Bromo- and nitro-phenylhydrazines, and asymmetrically disubstituted hydrazines of the type $NH_2 . NR . C_6H_5$, such as methylphenyl, benzylphenyl, diphenyl or $\beta$-naphthyl hydrazines, also react with the sugars, and some of these hydrazones are sparingly soluble and are characteristic of a particular sugar.

Thus the methylphenyl and dibromophenyl hydrazone is characteristic of galactose and the diphenyl hydrazone of arabinose. The influence of the position of the OH groups on the physical properties is even more marked in the case of the dihydrazones formed with diphenylmethane dimethyl dihydrazine, $CH_2[C_6H_4NMe . NH_2]_2$ (v. Braun).[28] Hydrazones are only produced when at least two or three of the hydroxyl groups attached to the carbon atoms immediately adjacent to the aldehydic group have the same spatial configuration. Thus, *d*-fucose, *l*-fucose, mannose, galactose, ribose, lyxose, arabinose, and rhamnose give hydrazones with this reagent, whilst glucose and xylose do not. It is possible that the reagent may be useful in deciding questions of configuration of the aldoses in view of this peculiarity.

Characteristic *m*-tolyl hydrazones were obtained from arabinose, rhamnose, fucose, galactose and mannose but not from xylose and *d*-fructose.

In the following table the melting-points of those hydrazones and osazones of the monosaccharides, which have proved most useful in practice for their isolation and characterisation, are listed.

An exhaustive list of these compounds is given by Vogel and Georg.

To prepare the phenyl osazone, glucose is heated with a considerable excess of phenyl hydrazine * (3-4 mols.) and acetic acid, the vessel being immersed in rapidly boiling water for an hour or more,

* It is important that the phenyl hydrazine should be almost colourless and free from oxidation products.

| | Glucose. | Mannose. | Fructose. | Galactose. | Arabinose. | Xylose. | Ribose. | Rhamnose. |
|---|---|---|---|---|---|---|---|---|
| *Hydrazones.* | | | | | | | | |
| Phenyl hydrazone | | 199°–204° | | | | | | |
| *m*-Nitro phenylhydrazone | | 166° | | 181° | | 130° | | |
| *p*-Nitro phenylhydrazone | 189° | 201° | 180° | 194° | | | | 190° |
| *p*-Bromo phenylhydrazone | | 208° | | | 168° | | 170° | |
| Methyl phenylhydrazone | | 181° | | 190° | 165° | | | |
| Benzyl phenylhydrazone | | | | | 174° | | | |
| Di-phenylhydrazone | | | | | 218° | | | |
| *Osazones.* | | | | | | | | |
| Phenylosazone | 210° | 210° | 210° | 184° | 166° | 163° | 166° | 182° |
| *p*-Nitro phenylosazone | 252° | | 252° | | | | | |
| *p*-Bromo phenylosazone | 215° | | | | | | | 218° |

when the insoluble osazone separates: it is best purified by crystallisation from a dilute solution of pyridine. The excess of phenyl hydrazine acts as an oxidising agent towards the phenyl hydrazone, converting the penultimate — CH(OH) group into — CO and being itself reduced to aniline and ammonia. The CO group so formed interacts with a further molecule of phenyl hydrazine to form the osazone—

| | | | |
|---|---|---|---|
| CHO | CH : N . NHPh | CH : N . NHPh | CH : N . NHPh |
| \| | \| | \| | \| |
| CH(OH) | CH(OH) | CO | C : N . NHPh |
| \| | \| | \| | \| |
| CH(OH) | CH(OH) | CH(OH) | CH(OH) |
| \| | \| | \| | \| |
| CH(OH) | CH(OH) | CH(OH) | CH(OH) |
| \| | \| | \| | \| |
| CH(OH) | CH(OH) | CH(OH) | CH(OH) |
| \| | \| | \| | \| |
| $CH_2(OH)$ | $CH_2(OH)$ | $CH_2(OH)$ | $CH_2(OH)$ |

Aldose —→ Hydrazone —→ Oxidation product —→ Osazone.

Glucose, mannose and fructose yield the same phenyl osazone, since the asymmetry of carbon 2 is destroyed in its formation. The osazones of the different sugars are as a class very similar in properties, those formed by the disaccharides being distinguished by their greater solubility in boiling water. The melting-points of the osazones depend very largely on the rate of heating and on the method of purification adopted, and too much dependence is not to be placed on them in identifying unknown sugars. Fischer, for example, states that carefully purified glucosazone heated rapidly in a narrow capillary tube begins to melt at 208° (corrected), and completely melts at this temperature with decomposition if the source of heat be withdrawn. When heating is continued at the same rate

the thermometer rises to 213° before the glucosazone completely melts. When the heating is slower the substance begins to sinter and melt at 195°. In the case of the disaccharides, where the purification of the osazone is more difficult, the determination of the exact melting-point is even less reliable.

The asymmetrically disubstituted hydrazines do not easily form osazones with glucose on account of their being unable to act as oxidising agents. Fructose is more easily attacked by them, and yields the same substituted osazones as would be obtained from glucose.

Directions for obtaining a maximum yield of glucosazone by Fischer's method have been given by Taketomi and Miura.[29] The ratio of crystallised sodium acetate to phenyl hydrazine hydrochloride should be 1 to 2·7. The yield increases with the amount of the reagent used up to 3·2 grams of phenyl hydrazine hydrochloride in 20 c.c. of water: the reaction is not completed within less than three hours, but further heating brings about decomposition.

It is often a matter of considerable difficulty to obtain a carbohydrate in a pure state from solutions which may also contain inorganic salts or nitrogenous substances. One of the methods adopted is to isolate the phenyl hydrazone, purify this by crystallisation, and decompose it into sugar and phenyl hydrazine. Fischer originally used fuming hydrochloric acid to effect the decomposition. Benzaldehyde was substituted for this by Herzfeld; the phenyl hydrazone is boiled in water with a slight excess of benzaldehyde, and the phenyl hydrazine removed from solution as insoluble benzaldehyde phenyl hydrazone,

$$C_6H_{12}O_5 : N . NHPh + C_6H_5 . CHO = C_6H_{12}O_6 + C_6H_5CH : N . NHPh$$

This method was repeatedly adopted with success by Fischer, but it gives less satisfactory results with the disubstituted hydrazones, in which case formaldehyde may with advantage be substituted for benzaldehyde, as suggested by Ruff and Ollendorf. The hydrazone is dissolved in dilute formaldehyde and heated at the temperature of the water bath,

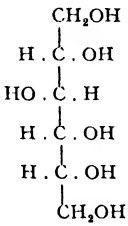
$$C_6H_{12}O_5 : N . NRR' + HCHO = C_6H_{12}O_6 + H . CH : N . NRR'$$

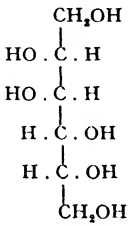
The excess of formaldehyde is removed and the pure sugar solution concentrated in a vacuum.

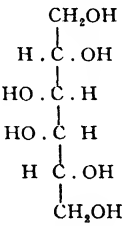
Nitrobenzaldehyde which forms a less soluble hydrazone than benzaldehyde has also been used.[30]

## Reduction.

When reduced with sodium amalgam, glucose and its isomerides form the corresponding hexahydric alcohols, two hydrogen atoms being added to the hexose. Sorbitol is formed from glucose, mannitol from mannose, and dulcitol from galactose. Fructose yields a mixture of the two alcohols, sorbitol and mannitol, since a new asymmetric carbon atom is formed from the ketonic radical. These alcohols have the following configurations :—


Sorbitol.


Mannitol.


Dulcitol.

All these alcohols are sweet, well-crystallised compounds quite soluble in water and alcohol. They form hexacetyl, hexabenzoyl and explosive hexanitro derivatives, also compounds with acetone and benzaldehyde.

Their configuration and natural distribution are discussed in detail in Chapter XIII.

The products of the electrolytic reduction [31] of glucose are formic acid and pentose : no hexahydric alcohol is formed.

The most effective way of obtaining the hexahydric alcohols from the hexoses is by catalytic hydrogenation under pressure.[32] The yields are quantitative, glucose giving sorbitol, and fructose almost entirely mannitol, together with a little sorbitol.

### REFERENCES TO CHAPTER V.—THE LOBRY DE BRUYN CONVERSION.

1. Lobry de Bruyn and Van Ekenstein, Rec. trav. chim., 1895, **14**, 156 ; 204. Van Ekenstein and Blanksma, Rec. trav. chim., 1908, **27**, 1.
2. Wohl and Neuberg, Ber., 1900, **33**, 3095.
3. Wolfrom and Lewis, J.A.C.S., 1928, 837.
4. Spoehr and Strain, J.B.C., 1929, **85**, 370.
5. Benedict, Dakin and West, J.B.C., 1926, **68**, 1.
6. Spoehr and Wilbur, J.B.C., 1926, **69**, 421.
7. Montgomery and Hudson, J.A.C.S., 1930, 2101.
8. Austin, J.A.C.S., 1930, 2106.
9. Smith, Biochem. J., 1932, **26**, 1467. Condrea and Roth, Compt. rend. Soc. Biol., 1933, **112**, 1497.

## ACTION OF STRONG ALKALIS.

10. KILIANI, Ber., 1908, **41**, 469.
11. NEF, Ann., 1907, **357**, 301.
12. FISCHLER, Z. Physiol. Chem., 1927, **165**, 53.
13. NEF, Ann., 1907, **357**, 214; Ann., 1910, **376**, 1; Ann., 1914, **403**, 204.
14. SOBOTKA, J.B.C., 1926, **69**, 267.
15. WILLSTÄTTER AND SCHUDEL, Ber., 1918, **51**, 780.

## ACTION OF AMMONIA.

16. LING AND NANJI, J. Soc. Chem. Ind., 1922, **41**, 151 T; J.C.S., 1922, 1682.
17. WINDAUS AND KNOOP, Ber., 1905, **38**, 1166.
18. WINDAUS, Ber., 1906, **39**, 3886; Ber., 1907, **40**, 799.
19. WINDAUS AND ULLRICH, Z. Physiol. Chem., 1924, **90**, 366.
20. PARROD, Ann. Chim., 1933 (10), **19**, 205.
21. LOBRY DE BRUYN, Rec. trav. chim., 1899, **18**, 72.
22. STOLTE, Hofm. Ber., 1908, **11**, 20.
23. PERVIER AND GORTNER, Ind. Eng. Chem., 1923, **15**, 1167; 1255.

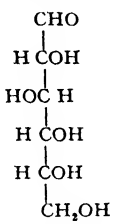
## ACTION OF PHENYL HYDRAZINE.

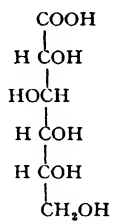
24. FISCHER, Ber., 1884, **17**, 579; Ber., 1887, **20**, 821; Ber., 1888, **21**, 988, 2631; Ber., 1889, **22**, 87.
25. SKRAUP, Monatsh., 1889, **10**, 406.
26. BEHREND AND LOHR, Ann., 1907, **353**, 106; Ann., 1908, **362**, 78; Ann., 1910, **377**, 189.
27. FRÈRE-JACQUE, Compt. rend., 1925, **180**, 1210.
28. V. BRAUN, Ber., 1917, **50**, 42. V. BRAUN AND BAYER, Ber., 1925, **58**, 2215.
29. TAKETOMI AND MIURA, J. Soc. Chem. Ind., Japan, 1929, **32**, 776.
30. VOTOČEK AND VALENTIN, Chem. Abstracts, A., 1931, 1274.
31. FINDLAY, Trans. Faraday Soc., 1922, **17**, 453.
32. IPATIEW, Ber., 1912, **45**, 3255. CAKE, J.A.C.S., 1922, 859. LLOYD, CONNOR AND ADKINS, J.A.C.S., 1932, 1651.

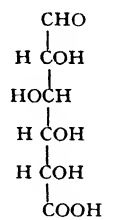
## CHAPTER VI.

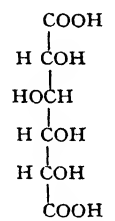
### THE SUGAR ACIDS.

By oxidation of the terminal groups on carbons 1 and 6 of the hexoses a number of different products are possible. From glucose are derived the following different acids :—


Glucose.


*d*-Gluconic.


*d*-Glucuronic.


*d*-Saccharic.

## Gluconic Acid.

In gluconic acid the potentially aldehyde group of glucose is oxidised to carboxyl. The oxidation can be achieved by means of hypobromites and this method is used for quantitative estimation of aldoses. For preparative purpose the oxidation is performed with bromine in acid or neutral solution.[1] It is convenient to carry out the oxidation in the presence of barium benzoate [2]; insoluble barium gluconate is precipitated and the destructive action of the hydrogen bromide is avoided.

All the aldohexoses behave similarly to glucose, but fructose and other ketose sugars are more resistant and are not oxidised by bromine water. This affords a method of differentiating between an aldose and a ketose.

A very convenient method is electrolytic oxidation [3] in presence of calcium bromide and calcium carbonate (to neutralise the acid produced). The electrolytically produced bromine effects the oxidation which is nearly quantitative, yielding crystalline calcium gluconate

The method can also be used for preparing galactonic and mannonic acids.[4]

Glucose is oxidised to gluconic acid by many bacteria and mould fungi. It was first isolated from *Aspergillus niger* cultures by Molliard [5] together with oxalic and citric acids. To give but a few examples, it is produced by *Citromyces glaber* and *P. glaucum* [6] and by *P. chrysogenum* and *Fumago vagans*.[7]

In the U.S.A. large quantities of gluconic acid are produced on a commercial scale, free from undesirable products, by means of *Penicillium luteum purpurogenum*.[8] The yield of acid is affected by the ratio of surface to volume of liquid, the best ratio being between 0·25 and 0·3. The mould is grown in shallow aluminium pans placed in a sterilisable chamber. As much as 80 per cent. of the theoretical amount is obtained. *d*-Mannonic acid was obtained [9] in a 9 per cent. yield from mannose by means of the same *P. purpurogenum var. rubrisclerotium*. It is of interest that this organism does not produce an acid from *d*-galactose.

The work of Raistrick and his collaborators [10] makes it appear that the first stage in the breakdown of the glucose molecule by fungi is a Cannizzaro reaction causing the formation from 2 molecules of glucose of 1 molecule of mannitol and 1 molecule of gluconic acid. Subsequently one or the other of these is destroyed, depending whether the particular mould prefers to grow in an acid or an alkaline medium. It is emphasised by Raistrick that careful search has failed to find sorbitol or mannonic acid as metabolic products of fungi.

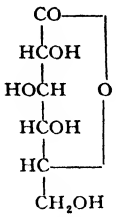
Gluconic acid and its lactones are of commercial importance in baking powders, whilst calcium gluconate is being increasingly used for the medical treatment of calcium deficiency.

Reference has been made to the use of the insoluble calcium and barium salts for isolation of gluconic acid. Strychnine and brucine salts and phenyl hydrazides are also used for the characterisation of aldonic acids.

Gluconic acid can be obtained crystalline by seeding a solution which is evaporated on the water bath. It is, however, easily changed over into the $\gamma$-lactone. Most of the aldonic acids are very readily converted into their crystalline $\gamma$-lactones.

Nef [11] was able to prepare the crystalline $\delta$-lactones of both mannonic and gluconic acid. Two different crystalline lactones of both glucose and mannose are therefore known.[12] In an aqueous solution of gluconic acid an equilibrium is reached between the parent

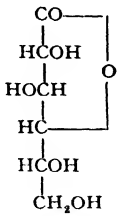
acid and the two lactones. The $\gamma$-lactones are more stable and much more slowly hydrolysed, and with $\gamma$-mannonolactone equilibrium is never reached under laboratory conditions. Their formulæ and properties are summarised below :—



1 : 5-Gluconolactone.

m.p. 152°.

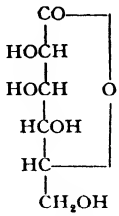
$[\alpha]_D$ +63·5°→6·2°. In 2½ hours.



1 : 4-Gluconolactone.

135°.

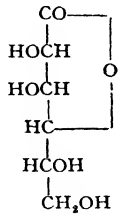
$[\alpha]_D$ +67·8°→58·7°. In 24 hours. →6·2°, Many days.



1 : 5-Mannonolactone.

156°.

$[\alpha]_D$ +114°→27·5°. In 22 hours.



1 : 4-Mannonolactone.

151°.

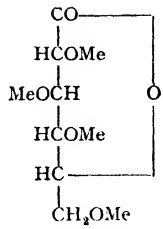
$[\alpha]_D$ +51·8°. No change in 24 hours.

The study of lactones has proved valuable for determining the configuration and ring structures of the sugars from which they are derived.

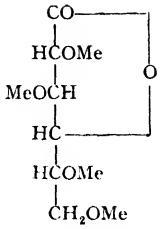
Hudson [13] in 1910 enunciated what is known as his "lactone rule," from the examination of the optical rotations of twenty-four sugar acid lactones which he assumed to be $\gamma$-lactones. He found that where the formation of the oxide ring engages a hydroxyl situated to the right on $C_4$ of the formula the lactone was dextrorotatory and conversely. On the other hand, if any ring other than a 5-ring was uniformly assigned to these lactones, no relations between configuration and rotation could be established. The critical assumption that all the lactones were $\gamma$-lactones was apparently justifiable.

The uses as well as the limitations of the rule have been discussed in Chapter IV. It cannot always be applied in the reverse sense to determine the ring structure of the lactone of a sugar of known configuration.

Haworth [14] and his collaborators have investigated the properties of the fully methylated lactones of sugar acids and isolated two lactones of widely different properties : one from the normal methylglucosides containing a 1 : 5 $\delta$-lactone ring, and a second from the labile methylglucofuranosides containing a 1 : 4 $\gamma$-lactone ring.



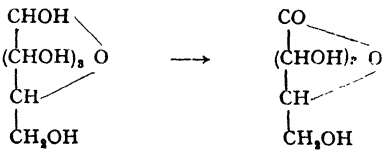
Tetramethyl 1 : 5 gluconolactone
from glucopyranoside.
Liquid.
Gives phenyl-hydrazide, m.p. 115°.



Tetramethyl 1 : 4 gluconolactone
from glucofuranoside.
Crystalline.
Gives phenyl-hydrazide,
m.p. 134°-136°.

Ten completely methylated lactones, derived from normal and γ-forms of completely methylated glucose, galactose, mannose, xylose, and arabinose have been studied by Drew, Goodyear and Haworth [15] in order to establish some correlation between the structure of these compounds and their behaviour on hydrolysis. It was found possible to discriminate sharply between the γ- and the δ-lactones. The δ-lactones are all rapidly hydrolysed, and the rotation reaches a constant equilibrium value in the course of a few hours. The γ-lactones are on the contrary very slowly hydrolysed, and many do not reach equilibrium values after several days. The two types of hydrolysis curves can be easily distinguished. Those lactones, which since they fit in with the Hudson rule are called γ-lactones, show the same hydrolytic behaviour as the fully methylated lactones of proved structure. Similarly, the δ-gluconolactone and δ-mannonolactone of Nef behave on hydrolysis like the fully methylated δ-lactones. The structure of these unsubstituted lactones is therefore confirmed.

Isbell and Pigman [16] have studied the mechanism of the oxidation of glucose with bromine water in weakly acid solutions saturated with carbon dioxide and buffered with barium carbonate. Under these conditions it was proved that glucose is directly oxidised to δ-gluconolactone, the 6-ring structure being preserved throughout. The δ-lactone is subsequently hydrolysed.



Glucose. δ-Gluconolactone.

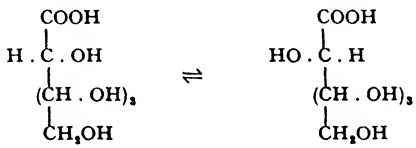
β-Glucose is oxidised about thirty-five times as rapidly as α-glucose.

Isbell and Pigman were able to calculate the proportion of α-glucose in the equilibrium mixture after mutarotation as 36 per cent., but it is not permissible to calculate the amount of β-form by difference, although from the evidence the mixture does consist substantially of these two forms. Galactose is more rapidly oxidised than glucose, an indication of the influence of the configuration upon reactivity.

Isbell [17] has also shown that in the oxidation of α- and β-mannose, α- and β-*l*-rhamnose, α- and β-lactose and β-maltose, the formation of the δ-lactone precedes that of the free acid. There is no evidence therefore that the oxidation of the normal aldoses proceeds through the free aldehyde form of the sugar as has sometimes been supposed. The experiments also confirm the pyranose structure of the parent sugars.

## Epimerisation.

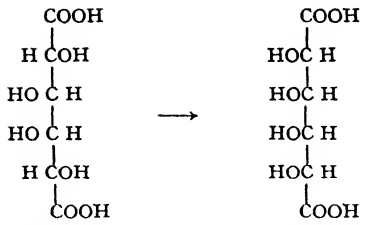
An important property of gluconic and the other aldonic acids, and one which has been of great value in effecting the synthesis of the sugars, is their behaviour on heating with quinoline or pyridine. When gluconic acid is so heated at 130°-150°, it is partially converted into mannonic acid. The rearrangement is apparently restricted to the groups attached to $C_2$, as in the interconversion of glucoss and mannose by weak alkalis. It is reversible, mannonic acid being converted into gluconic acid :—



*d*-Gluconic acid. *d*-Mannonic acid.

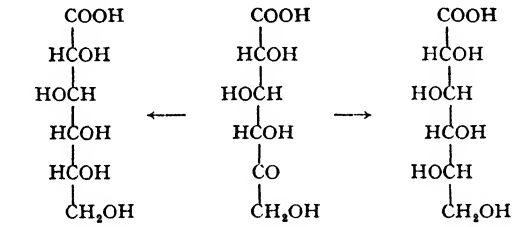
Similarly, *d*-galactonic and *d*-talonic acid are mutually interconvertible.

The fully methylated γ- and δ-lactones derived from the sugar pairs glucose and mannose, xylose and lyxose, are interconvertible by epimerisation in aqueous pyridine.[18] Dibasic sugar acids can also be epimerised, both ends of the molecule being affected. Thus if ammonium mucate is heated at 135° in aqueous solution it is largely converted into allomucic acid :—[19]



Mucic acid. Allomucic acid.

When glucose or gluconic acid are oxidised either by *B. xylinum* [20] or gluconic acid by cold nitric acid,[21] 5-ketogluconic acid (also known as *l*-sorburonic acid) is formed. This on partial reduction gives rise to two aldonic acids and subsequently to two aldoses :—



*d*-Gluconic acid. 5-Ketogluconic acid. *l*-Idonic acid.

This procedure affords a means of inverting the groups on $C_5$

## Glucuronic Acid.*

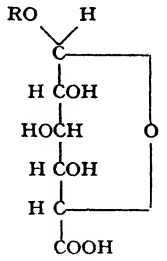
Physiologically the most interesting oxidation product of glucose is glucuronic acid, which is frequently found in the urine in combination with a variety of substances.

The animal organism has the power of combining substances which are toxic, or which can only be oxidised slowly, with glucuronic acid and excreting them in the urine. It was once suggested that the substances first form a glucoside with glucose, which then, since the aldehyde group is protected, is oxidised at the other end of the chain. This can no longer be upheld since Pryde [22] has shown that phenyl and bornyl-$\beta$-glucosides are not converted by the dog to the corresponding glucuronates. The source of glucuronic acid is probably mucin.

Compounds which have alcoholic or phenolic groups form compounds of glucosidic type, combining with the aldehyde group : others,

* Also written glycuronic acid.

such as chloral, are first reduced to the corresponding alcohol before combining.




According to da Cruz,[23] menthol glucuronate is hydrolysed by emulsin, and therefore is a β-glucuronoside. On the other hand, Miwa [24] reports that an enzyme which hydrolyses two flavone glucuronates, known as baicalin and scutellarin, found in *Scutellaria* species, has no action upon menthol glucuronate nor on β-glucosides, and that emulsin has no action upon baicalin or scutellarin. According to Helferich the enzyme in emulsin which hydrolyses glucuronates cannot be identified with β-glucosidase, and is probably a separate enzyme.

Pryde and Williams [25] conclude from methylation experiments that borneol glucuronate contains a pyranose ring. According to Quick,[26] glucuronic acid can be most conveniently prepared from borneol glucuronate. Borneol can be administered in 5-gram doses daily for weeks to a dog without ill-effects and a 50 per cent. recovery of borneol as glucuronate is obtained, the compound being precipitated as its zinc salt from the acidified urine.

Glucuronic acid and also the conjugated glucuronates such as the menthol compound can be burned by the body, and the conjugate appearing in the urine represents the fraction which has escaped complete oxidation. The output of glucuronic acid is greatly increased by the administration of insulin. The place of glucuronic acid in the general scheme of detoxication mechanisms of the body has been studied by Quick.[27]

With benzoic acid and other acids, conjugation with glucuronic acid also occurs through the aldehyde group. Glucuronic acid monobenzoate shows mutarotation, reduces Fehling's solution, combines with hydrogen cyanide and forms a methyl glycoside, but in these reactions the benzoyl group is hydrolysed.[28] *p*-Hydroxy benzoic acid forms a diglucuronate, 1 molecule of glucuronic acid being in

true glycosidic union with the phenolic hydroxyl and the second being joined to the carboxyl group as in the monobenzoate.[29]

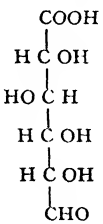
*d*-Glucuronic acid can conveniently be prepared from the hydrolysis products of gum-arabic according to Weinmann [30] in 5 per cent. yield. Glucuronic acid is an important constituent of the plant gums and of hemicelluloses, in the latter it stands midway between the cellulose and the xylan.

An interesting property of glucuronic acid is its ready decarboxylation to yield xylose which, according to Franken,[31] occurs on heating in 4 per cent. sulphuric acid : owing to the simultaneous production of furfural, the yield is poor. Neuberg and Salkowski [32] observed that bacteria from decaying flesh were able to decarboxylate glucuronic acid to *d*-xylose.

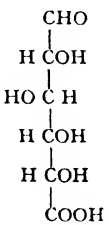
*d*-Glucuronic acid is found in the type specific polysaccharide of type III. pneumococcus in combination with glucose, and a similar substance, *d*-galactopyranose-6-glucuronate, is a partial hydrolysis product of gum-arabic.

Another biological oxidation product of glucose is "*l*-glucuronic acid," 6-aldehydo-gluconic acid. It is not the true antimer of *d*-glucuronic acid and should properly be called *l*-guluronic acid.

It is prepared by the action of *B. industrium* on a calcium gluconate medium in 25 per cent. yield.[33]



6-Aldehydogluconic
= "*l*-Glucuronic" acid.

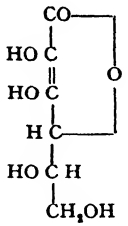


*d*-Glucuronic acid.

## Ascorbic Acid.

Szent-Györgyi,[34] through an investigation of biological oxidation systems, was led to discover a substance $C_6H_8O_6$, first in the adrenal cortex and later as widely distributed in animal and plant sources. The best source so far discovered is paprika. The name hexuronic acid first given to the substance has been altered to ascorbic acid.

According to the work of Hirst, Haworth [35] and others, ascorbic acid is a lactone and is the enolic form of 3-keto-*l*-gulonolactone.

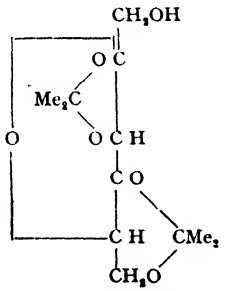


Its synthesis has been achieved starting from *l*-xylosone.[36]

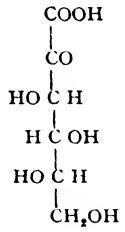
Ascorbic acid is very important biologically, much evidence existing that it is identical with the antiscorbutic vitamin C, and is in fact this vitamin in crystalline condition.[37]

As it stands, the configuration of ascorbic acid does not permit it to be derived simply from the naturally occurring hexoses, except *l*-sorbose which is derived from *d*-glucose and is formed by certain oxidising bacteria from sorbitol, the alcohol formed by reduction of glucose.

This relationship forms the basis of the synthesis of ascorbic acid starting from glucose, by Reichstein.[38] Glucose is converted to sorbitol by reduction, and this by oxidation with *B. Xylinum* to sorbose. Sorbose diacetone on permanganate oxidation yields the diacetone derivative of 2-keto-*l*-gulonic acid, and the free acid, $[\alpha]_D$ — 48°, m.p. 171°, on treatment with acids, is converted smoothly into the isomeric *d*-ascorbic acid.



Diacetone-sorbose.



2-Keto-*l*-Gulonic acid.

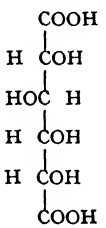
Investigation has shown that iris leaves and other such sources contain as much as 0·3 per cent. of ascorbic acid, and therefore it is to be regarded as an important carbohydrate constituent of plants.

*d*-Mannuronic acid has been obtained from the polymeric alginic acid of *Laminaria saccharina* and *Fucus serratus* by Nelson and Cretcher.[39] *d*-Galacturonic acid is an important constituent of pectin and of gums and mucilages and is also found in hemicelluloses. It is conveniently prepared from lemon pectin.[40] It is decarboxylated to *l*-arabinose by heating in 2 per cent. sulphuric or oxalic acid (Franken).

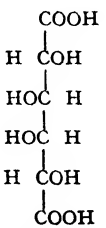
Saccharic acid is formed by the action of nitric acid on glucose; it forms a sparingly soluble acid potassium salt which serves as a test for glucose. Saccharic acid is also produced by nitric acid oxidation from sucrose, raffinose, dextrin and starch. Some saccharic acid is produced by *Aspergillus niger* grown on glucose cultures. On the other hand, mucic acid, the corresponding oxidation product of galactose, is obtained by the action of nitric acid on galactose, dulcitol, lactose, melibiose, and the pectins.

Free saccharic acid was obtained crystalline by Rehorst;[41] in solution it forms an equilibrium with the monobasic $\gamma$-lactonic acid, the rotatory power rising from 6·9° to 21·4°. The lactone crystallises in leaflets; in solution the rotation falls from 37·9° to 21·4° as it is partially converted into the free acid.

Mucic acid has been obtained crystalline and is optically inactive. When it is dry distilled pyromucic acid (furane $\alpha$-carboxylic acid) is formed: heating with hydrobromic acid converts it into dehydromucic acid (furane-$\alpha\alpha'$-dicarboxylic acid). It is manufactured from the wood of the Western larch, which yields 10 per cent. of a water soluble galactan made up of 85 per cent. galactose and 12 per cent. arabinose; it is used for various purposes as a substitute for the more expensive tartaric acid.




*d*-Saccharic acid.




Mucic acid.

## REFERENCES TO CHAPTER VI.—THE SUGAR ACIDS.


1. KILIANI AND KLEEMAN, Ber., 1894, **17**, 1296. HERZFELD AND LENARD, Zeit. ver. Deut. Zuckerind., 1919, 122.
2. HUDSON AND ISBELL, J.A.C.S., 1929, 2225.
3. ISBELL AND FRUSH, Bur. Stand. J. Res., 1931, **6**, 1145.
4. KILIANI, Ber., 1932, **65**, 1269; Ber., 1933, **66**, 117.
5. MOLLIARD, Compt. rend., 1922, **174**, 881.
6. BUTKEWITSCH, Biochem. Z., 1927, **182**, 99.
7. BIRKINSHAW AND RAISTRICK, Phil. Trans., 1931, **220 B**, part XVII.
8. MAY, HERRICK, MOYER AND HELLBACH, J.B.C., 1928, **77**, 185; J. Ind. Eng. Chem., 1929, **21**, 1198.
9. ANGELETTI AND CERRUTTI, Ann. Chim. Appl., 1930, **20**, 424.
10. RAISTRICK et alli, Phil. Trans., 1931, **220 B**.
11. NEF, Ann., 1914, **403**, 204.
12. HAWORTH AND NICHOLSON, J.C.S., 1926, 1899.
13. HUDSON, J.A.C.S., 1910, 338.
14. CHARLTON, HAWORTH AND PEAT, J.C.S., 1926, 89. HAWORTH, HIRST AND MILLER, J.C.S., 1927, 2436.
15. GOODYEAR AND HAWORTH, J.C.S., 1927, 1237.
16. ISBELL AND PIGMAN, Bur. Stand. J. Res., 1933, **10**, 337.
17. ISBELL, Bur. Stand. J. Res., 1932, **8**, 615.
18. HAWORTH AND LONG, J.C.S., 1929, 345.
19. S. AND T. POSTERNAK, Helv. Chim. Acta, 1929, **12**, 11.
20. BERTRAND, Ann. Chim. Phys., 1904, **3**, 181.
21. KILIANI, Ber., 1922, **55**, 79; 2822.
22. PRYDE, Biochem. J., 1934, **28**.
23. DA CRUZ, Compt. rend. Soc. Biol., 1930, **105**, 815.
24. MIWA, Acta Phytochim., 1932, **6**, 155.
25. PRYDE AND WILLIAMS, Biochem. J., 1933, **27**, 1197.
26. QUICK, J.B.C., 1927, **74**, 331.
27. QUICK, J.B.C., 1932, **95**, 189.
28. QUICK, J.B.C., 1926, **69**, 549.
29. QUICK, J.B.C., 1932, **97**, 403.
30. WEINMANN, Ber., 1929, **62**, 1637.
31. FRANKEN, Biochem. Z., 1932, **250**, 53.
32. NEUBERG AND SALKOWSKI, Z. Physiol. Chem., 1902, **36**, 261.
33. TAKAHASHI AND ASAI, Zentr. Bakt. Par., 1931, **84**, 193.
34. SZENT-GYÖRGYI, Biochem. J., 1928, **22**, 1389
35. HERBERT, HIRST, PERCIVAL, REYNOLDS AND SMITH, J.C.S., 1933, 1270.
36. HAWORTH, HIRST ET ALII, J.C.S., 1933, 1419.
37. SZENT-GYÖRGYI, Nature, 1933, **131**, 225.
38. REICHSTEIN AND GRÜSSNER, Helv. Chim. Acta, 1934, **17**, 311.
39. NELSON AND CRETCHER, J.A.C.S., 1932, 3409.
40. LINK AND DICKSON, J.B.C., 1930, **86**, 491.
41. REHORST, Ber., 1928, **61**, 163.

## CHAPTER VII.

### THE SYNTHESIS OF THE MONOSACCHARIDES BY CHEMICAL MEANS.

THE synthetical preparation of natural dextro-glucose from its elements may be justly claimed as one of the greatest achievements of the chemist.

In the following section a brief outline is given of the operations performed in preparing glucose and fructose from their elements. The first attempt which was in any way successful was that made by Butlerow [1] in 1861, who showed that when formaldehyde is condensed by means of lime water a syrupy substance is obtained which has the properties of a sugar. Subsequently Loew [2] improved the technique of the method and named the product he obtained formose. Fischer and Tafel,[3] starting with acrolein dibromide, effected condensation by means of baryta, the change being expressed by the equation

$$2C_3H_4OBr_2 + 2Ba(OH)_2 = C_6H_{12}O_6 + 2BaBr_2$$

They showed that the syrupy product obtained contained two sugars distinguished as $\alpha$- and $\beta$-acrose. Subsequently glycerose was made the starting-point for the synthesis; crude glycerose is a mixture of glyceric aldehyde, $CH_2(OH) . CH(OH) . CHO$, and dihydroxyacetone, $CH_2(OH) . CO . CH_2(OH)$, and these two compounds can be formulated as undergoing the "aldol" condensation, forming a ketose, $CH_2(OH) . (CH . OH)_3 . CO . CH_2(OH)$, which has the same formula as fructose. $\alpha$- and $\beta$-acrose were also obtained from this condensation, and characterised by means of the osazones they formed with phenylhydrazine. $\alpha$-Acrosazone was found to possess a remarkable resemblance to glucosazone, differing only in being optically inactive. Fenton [4] has shown that glycollic aldehyde, $CH_2(OH) . CHO$, may be used as the starting-point of the synthetical process; three molecules of it condense to $\alpha$-acrose.

A product of synthesis by all these methods is $\alpha$-acrose. Fischer [5, 6] converted this firstly into acrose phenylosazone in order to isolate

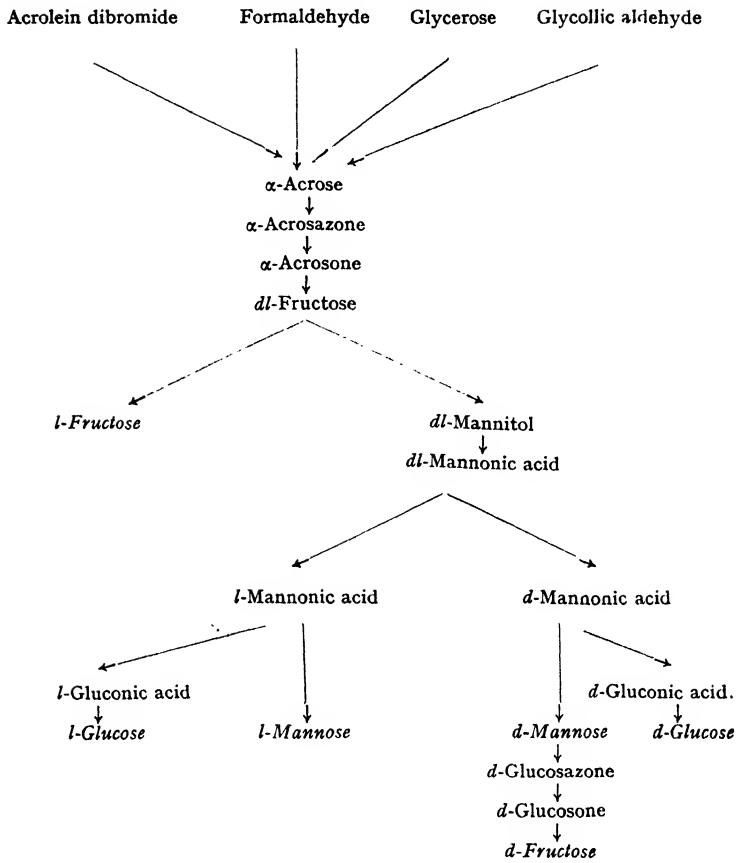
it from the mixture of other substances and then into acrosone by treatment with hydrochloric acid. Acrosone, on reduction, yielded firstly a sweet syrup having all the properties of fructose, and secondly on further reduction an alcohol, α-acritol, very like mannitol but differing in being optically inactive. There was no doubt that α-acrose was inactive *dl*-fructose. The further problem was to obtain an optically active sugar from this. The product was partially fermented with yeast and a dextrorotatory sugar, *l*-fructose, was obtained, but this biological method did not lead to the isolation of the natural sugar. To obtain this a number of operations were necessary. *dl*-Fructose was reduced to *dl*-mannitol and the latter oxidised to the corresponding acid, *dl*-mannonic acid. (This acid forms a characteristic hydrazide from which it can be easily regenerated.) The racemic acid gave crystalline alkaloid salts and these were separated by fractional crystallisation; in this manner their resolution into the optically active forms was effected just as was done by Pasteur with racemic tartaric acid. *d*- and *l*-mannonic acids were thus obtained by the crystallisation of the strychnine or morphine salt of the synthetical racemic acid: by reduction of their lactones, they were converted into *d*- and *l*-mannose and the complete synthesis of these hexoses accomplished. To pass to *d*-fructose it only remained to reduce the mannosone (identical with glucosone) formed from *d*-mannose phenylosazone in the manner already described.

The synthetical mannonic acids are converted into the corresponding gluconic acids when heated with pyridine or quinoline, and it was only necessary to reduce these acids to obtain the corresponding glucoses. The stages of these syntheses are summarised in the chart on the next page.

Proceeding in this way Fischer effected the synthesis of the six hexoses derived from mannitol, and extended the methods to the synthesis of a number of isomeric hexoses which do not occur naturally. To-day, out of the sixteen isomeric aldohexoses possible, according to the Le Bel-Van't Hoff theory, all sixteen have been prepared synthetically.

Fischer regarded the other products of synthesis as either allied to sorbose or containing a branched and not a straight chain of carbon atoms. Nef stated that formose consists of hexoses and pentoses in equal proportions.

β-acrose has been shown to be *dl*-sorbose by Küster and Schoder.[7] There is also present in the formose mixture a ketopentose.



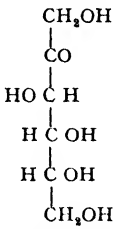

By alkaline condensation of pure glyceraldehyde under conditions which would be unlikely to cause the aldose ⇌ ketose conversion (presence of excess of 0·1 per cent. baryta at the ordinary temperature), Schmitz [8] obtained a solid crystalline mixture of inactive hexoses. Recrystallisation from hot methyl alcohol separated this into *dl*-fructose (α-acrose), m.p. 129°-130°, and *dl*-sorbose, m.p. 162°-163°, which represents β-acrose. The appearance of the ketonic group in the sugar synthesis must take place at the triose stage and therefore, strictly speaking, the reaction is condensation of glyceraldehyde with dihydroxyacetone and not auto-condensation of glyceraldehyde: limitations are thus imposed on the number of hexoses which can theoretically be produced. The mechanism of acrose formation is thus established with a considerable degree of certainty.

Both glycollic aldehyde and dihydroxyacetone are produced when formaldehyde is condensed by means of calcium carbonate, and H. and A. Euler [9] have shown that a pentose, *dl*-arabinoketose, is the main product of this polymerisation. It is derived from the condensation of glycollic aldehyde and dihydroxyacetone :—

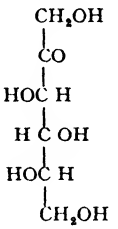
$$CH_2(OH).CHO + CO(CH_2.OH)_2 = CH_2(OH).(CH.OH)_2.CO.CH_2(OH)$$

Arabinoketose has not yet been found among plant products.

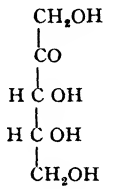
The space formulæ for the synthetic sugars are



*d*-Fructose.



*l*-Sorbose.



*d*-Arabinoketose.

Theoretically a simpler method of passing from *dl*-fructose (α-acrose) to glucose and mannose is afforded by warming with alkali, when the transformations observed by Lobry de Bruyn take place to give an equilibrium mixture. These are of particular interest with sorbose, which is converted into galactose and tagatose. Sorbose on reduction gives mannitol and sorbitol of the glucose series, whereas the Lobry de Bruyn transformation converts it to the galactose series, so that this transformation connects the hexoses of the two main natural series.

Before this transformation was discovered Fischer found it necessary to degrade gulonic acid to the pentose sugar *d*-xylose, transform this into the isomeric *d*-lyxose, and combine lyxose with hydrogen cyanide to give *d*-galactonic acid. It was only in this somewhat roundabout fashion that the complete synthesis of galactose and other hexoses derived from dulcitol could be effected.

No one has yet been able to convert glucose into galactose in the laboratory, but the change takes place readily in the mammary gland.

Baly's [10] synthetic sugar is not α- and β-acrose. The crude syrup, according to Irvine and Francis,[11] contains no ketoses and some 26 per cent. of aldoses : the presence of glucose was not definitely proven. It is clear the polymerisation of formaldehyde by ultra-violet light and chemical means do not proceed in the same way.

## The Cyanhydrin Synthesis.

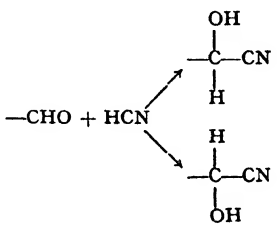
The laboratory methods for converting a sugar into one with a greater number of carbon atoms in the chain, for example, a pentose into a hexose, are of interest for they have been useful in establishing the configuration of the sugars. In nature a similar process probably operates to convert the hexoses into the rarely found heptoses, but it is not believed that the hexoses themselves are built up by the addition of one carbon atom at a time to the lower members of the series, but rather that they are synthesised at one step. The natural pentoses, arabinose and xylose are in all probability derived by the degradation of the hexoses by one carbon atom.

The aldoses combine directly with hydrogen cyanide forming nitriles; these, when hydrolysed, give the amides and finally the acids containing one carbon atom more than the original sugar. The lactones of these acids, when reduced with sodium amalgam, yield the corresponding aldoses with one carbon atom more than the original carbohydrate.

$$C_5H_{11}O_5 \cdot CHO + HCN \rightarrow C_5H_{11}O_5 \cdot CH(OH) \cdot CN \rightarrow C_5H_{11}O_5 \cdot CH(OH) \cdot CO_2H \rightarrow C_5H_{11}O_5 \cdot CH(OH) \cdot CHO$$

By this method, first discovered by Kiliani,[12] it is possible, adding one carbon atom at a time, to advance from formaldehyde to a biose and so on to the higher sugars. Fischer,[13] who made great use of this method, carried glucose and mannose up to aldononoses, galactose to galaoctose and rhamnose to rhamnoheptose. Philippe [14] has prepared glucodecose.

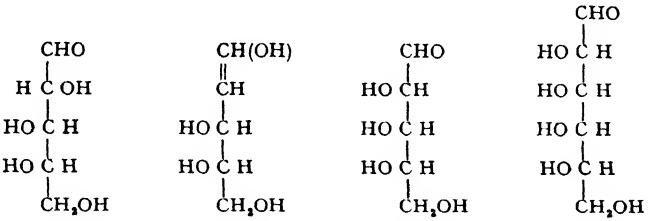
The cyanhydrin synthesis, however, is complicated by the fact that two stereoisomeric nitriles are formed simultaneously. Arabinose gives both glucose and mannose, indicating that the only difference in the structural configuration of these two hexoses must be in the second carbon atom; glucose yields two glucoheptoses. A new asymmetric carbon atom is created in the nitrile, and, according to theory, two forms will be produced unless the synthesis is asymmetric in character :—



Mannose affords the only instance at present recorded in which only one nitrile is formed to the practical exclusion of the other, but it is usual to find one form predominating.

Austin and Humoller [22] have recently synthesised *l*-allose and *l*-altrose, completing the series of the sixteen aldohexoses. *l*-arabinose was converted into acetobromoarabinose, and this by zinc dust reduction and deacetylation into arabinal, which, treated with perbenzoic acid, gave a mixture of *l*-arabinose and *l*-ribose.

*l*-Ribose by the cyanhydrin reaction gives a mixture of *l*-altronic and *l*-allonic acids, which can be separated by their copper salts. Sodium amalgam reduction of *l*-allonolactone yields *l*-allose $[\alpha]_D - 1{\cdot}9°$ mutarotating to $-13{\cdot}88°$, and of *l*-altronolactone yields *l*-altrose, $[\alpha]_D$ $-28{\cdot}75°$ mutarotating to $-32{\cdot}3°$.




*l*-Arabinose. Arabinal. *l*-Ribose *l*-Allose.

*d*- and *l*-allose, together with *d*- and *l*-glucose, *d*- and *l*-galactose, *d*- and *l*-mannose and *l*-altrose are the only aldohexoses which have been obtained crystalline.

Kiliani has extended the hydrogen cyanide synthesis to galactose in such a way as to add a new carbon atom at each end of the molecule. The product of the first addition is α-galactoheptonic acid, which on careful oxidation with warm nitric acid yields *l*-mannohepturonic acid, $CHO \cdot (CHOH)_5 \cdot CO_2H$, so named from its possessing the configuration of *l*-mannuronic acid for carbons 3-7. By addition of a further molecule of hydrogen cyanide, α-galaoctane hexol-diacid $CO_2H \cdot (CHOH)_6 \cdot CO_2H$ is obtained, to which, as it is optically inactive, a symmetrical formula is ascribed.

## The Degradation of a Sugar.

The conversion of a sugar into one with fewer carbon atoms is of equal importance to its conversion into a higher sugar for the determination of configuration. It has been achieved by several experimental methods.

In the method of Wohl [15] the oxime of the aldose is heated with concentrated sodium hydroxide and converted by dehydration into

the nitrile of the aldonic acid, from which, on further heating, hydrogen cyanide is eliminated and a pentose formed.

| CHO | CH = N . OH | CN + $H_2O$ | HCN |
|---|---|---|---|
| CH(OH) | CH(OH) | CH(OH) | CHO |
| Hexose. | Oxime. | Nitrile. | Pentose. |

In practice it is preferable to heat the oxime with acetic anhydride and sodium acetate, conditions which produce the acetylated nitrile, from which hydrogen cyanide is eliminated by treatment with ammoniacal silver oxide. A modification due to Zemplen [16] consists in the use of sodium methoxide in chloroform solution with the acetylated nitrile. Deulofeu,[17] who has made comparative studies of the different methods of degradation, recommends its use only for sugars higher than the pentoses and methylpentoses, because the concentration of alkali that is necessary for the reaction decomposes the lower members of the monose series and low yields of sugar are obtained. The methods of Wohl, Ruff and Weermann have general applicability.

The method due to Ruff [18] makes use of Fenton's mode of oxidation with hydrogen peroxide and ferrous salts. The aldose is first converted into aldonic acid, the calcium salt of which is subjected to oxidation, with the result that the carboxyl group is eliminated and the pentose formed :—

| CHO | $CO_2H$ | $CO_2 + H_2O$ |
|---|---|---|
| CH(OH) | CH(OH) | + |
| | | CHO |
| Aldohexose. | Aldonic acid. | Aldopentose. |

Neuberg [19] has used an electrolytic method : the aldose is converted into the corresponding acid, the copper salt of which is then electrolysed between platinum electrodes.

Weermann [20] has contributed another method of degradation. Taking glucose → arabinose, for example, *d*-gluconamide is produced by saturating an alcoholic solution of gluconolactone with ammonia, and this is treated with hypochlorous acid and caustic soda, when the following change occurs (analogous to the Hofmann reaction with hypobromite) :—

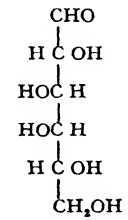
$$R . CH(OH) . CO . NH_2 \rightarrow R . CH(OH) . N : C : O \rightarrow R . CHO + HNCO$$

In this way a 50 per cent. yield of *d*-arabinose was obtained.

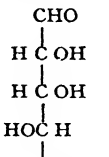
An interesting application of this method is afforded by the preparation of *l*-lyxose from *d*-galactose, carried out by Haworth

and Hirst in the course of the synthesis of *l*-ascorbic acid. The complete preparation involves the passage from the *d*- to the *l*-series by the turning round as it were of the galactose molecule. The transformations in order are—

*d*-galactose → *d*-galactose diacetone → *d*-galacturonic acid diacetone →*d*-galacturonic acid→*l*-galactonolactone→*l*-galactonamide→*l*-lyxose.



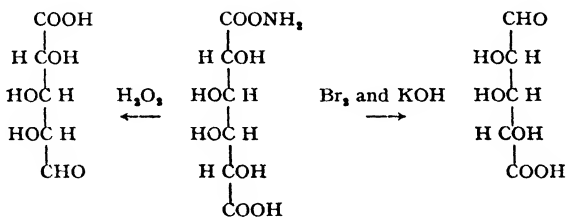

*d*-Galactose.




*l*-Lyxose.

The variety of methods makes it possible to degrade any aldose to the next lowest member of the series. Almost every sugar has been so degraded, with the result that every possible sugar from glycerose to the pentoses inclusive is known, and has been synthesised by this or other methods.

Bergmann [21] was able to degrade mucic acid at either end of the molecule by acting on the semiamide with different oxidising agents. Hydrogen peroxide oxidises the free carboxyl group, forming the amide of *l*-lyxuronic acid from which the free acid can be obtained. Bromine and potash oxidise the amide group, yielding *d*-lyxuronic acid.




The diamide of mucic acid can be degraded at both ends to yield the aldehyde of lævo tartaric acid, which can be isolated as its di-phenyl-hydrazone.

## REFERENCES TO CHAPTER VII.—SYNTHESIS OF THE MONOSACCHARIDES.


1. Butlerow, Compt. rend., 1861, **53**, 145; Ann., 1861, **120**, 295.
2. Loew, J. Pr. Chem., 1886, **33**, 321; Ber., 1889, **22**, 470.
3. Fischer and Tafel, Ber., 1887, **20**, 2566, 3384; Ber., 1889, **22**, 97.
4. Fenton, J.C.S., 1897, 375.
5. Fischer, Ber., 1890, **23**, 2114; Ber., 1894, **27**, 3189; Ber., 1890, **23**, 370, 799
6. Fischer and Passmore, Ber., 1899, **22**, 359.
7. Küster and Schoder, Z. Physiol. Chem., 1924, **141**, 110.
8. Schmitz, Ber., 1913, **46**, 2327.
9. H. and A. Euler, Ber., 1906, **39**, 39.
10. Baly, J.C.S., 1921, 1025.
11. Irvine and Francis, Ind. Eng. Chem., 1924, **16**, 1019.


### CYANHYDRIN SYNTHESIS.


12. Kiliani, Ber., 1886, **19**, 221, 3033; Ber., 1888, **21**, 915.
13. Fischer, Ann., 1892, **270**, 64; Ann., 1895, **288**, 139. Fischer and Passmore, Ber., 1890, **23**, 2226, 2433. Fischer and Piloty, Ber., 1890, **23**, 3102.
14. Philippe, Ann. Chim. Phys., 1912, **26**, 289.


### DEGRADATION OF A SUGAR.


15. Wohl, Ber., 1893, **26**, 730; Ber., 1897, **30**, 3101; Ber., 1899, **32**, 3666.
16. Zemplen, Ber., 1926, **59**, 1254. Zemplen and Kiss, Ber., 1927, **60**, 165.
17. Deulofeu, J.C.S., 1930, 2602.
18. Ruff, Ber., 1899, **32**, 550, 3672; Ber., 1901, **34**, 1362.
19. Neuberg, Biochem. Z., 1908, **7**, 527.
20. Weermann, Rec. trav. chim., 1917, **37**, 16.
21. Bergmann, Ber., 1921, **54**, 1362, 2651.
22. Austin and Humoller, J.A.C.S., 1932, 4749; J.A.C.S., 1933, 2167, 1934, 1153.

## CHAPTER VIII.

### ESTERS AND ETHERS OF GLUCOSE.

### The Methyl Glucoses.

THE methyl glucoses (*o*-methyl glucoses) are true ethers of the sugars in which $OCH_3$ replaces OH.

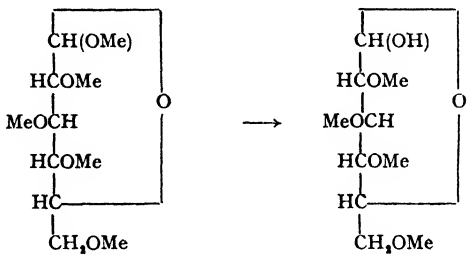
The great value of methylation has been that as far as is at present known stereochemical changes such as racemisation or Walden inversion, wandering of substituents and changes in ring structure do not take place. Many of the methyl glucoses can be purified by vacuum distillation and obtained crystalline. The methylated sugars have therefore proved of great value for determining the structure of the di- and polysaccharides as well as of the partially substituted sugars.

It was first shown by Purdie and Irvine [1] that it was possible to methylate the methyl glucosides by exhaustive treatment with methyl iodide and silver oxide. Haworth [2] worked out a second and less expensive method, involving the use of methyl sulphate and sodium hydroxide solution, which was first used by Denham and Woodhouse.[3]

The lactones of sugar acids are methylated in similar manner. The free sugars cannot be directly methylated, as silver oxide has an oxidising action towards the aldehyde group, but their methyl glycosides can be methylated in a solution of methyl alcohol up to the trimethyl stage $C_6H_8O_2(OCH_3)_4$. Further complete methylation is effected in methyl iodide solution.[4]

By Haworth's method (l.c.) the sugar dissolved in the least quantity of water is stirred at 70°, and in the course of an hour three times the theoretical quantity of the alkylating reagents are added from separate funnels; subsequently the temperature is raised to 100° for half an hour. Sometimes by this method also the last hydroxyl group is left wholly or incompletely methylated owing to the diminishing solubility of the products in the medium: resource must then be had to methyl iodide and silver oxide.

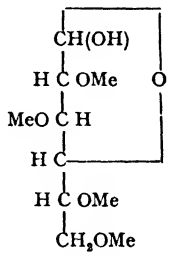
2 : 3 : 4 : 6-*Tetramethyl Glucose.*—The two isomeric pentamethyl glucoses or α- and β-tetramethyl-methyl glucosides are hydrolysed by dilute acids to the corresponding tetramethyl glucoses, the remaining four methoxyl groups being stable towards dilute acids.




Tetramethyl glucose undergoes mutarotation in solution : when tetramethyl glucose is etherified by Fischer's method a mixture of the α- and β-tetramethyl methyl glucosides is obtained.

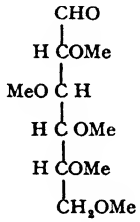
As already indicated, the pyranose formula for glucose is based on the proof of the structure of 2 : 3 : 4 : 6-tetramethyl glucose from its oxidation to *i*-trimethyoxyglutaric acid.

2 : 3 : 5 : 6-*Tetramethyl Glucose.*—This tetramethyl glucose is obtained as a syrup after methylation, and subsequent hydrolysis of methyl or ethyl glucofuranoside or of glucose monoacetone.[5] It yields on oxidation a characteristic γ-lactone, which on further oxidation with nitric acid yields dimethyl *d*-tartaric acid and oxalic acid : on these results depends the structure assigned to glucofuranose.



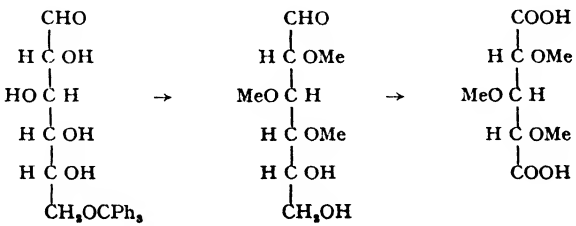

The α- and β-tetramethyl methyl glucofuranosides are best obtained by methylation of the α- and β- 3 : 5 : 6-trimethyl methyl glucosides.[6] They are syrups.

2 : 3 : 4 : 5 : 6-*Pentamethyl Glucose* was obtained as a syrup by Levene and Meyer [7] from its mercaptal, prepared by the methylation of diethyl mercaptoglucose.



The trimethyl hexoses and the lower partially methylated hexoses have proved important and useful substances. It is essential to know the structure of the trimethyl hexoses which are obtained by hydrolysis of the fully methylated di- and polysaccharides; the structure of these derivatives has been established both by degradation and by synthesis. The methods employed in their preparation consist in methylation of hexose derivatives in which certain of the hydroxyl groups are shielded from attack; for instance, the terminal (aldehydic) hydroxyl may be transformed to methyl glycoside before the operation, or other of the hydroxyl groups may be temporarily occupied in condensation complexes with compounds such as benzaldehyde or acetone. The primary alcohol group of $C_6$ can be protected by Helferich's method of forming the triphenylmethyl or trityl derivative. The partially methylated glucoses are obtained on submitting these compounds to hydrolysis.

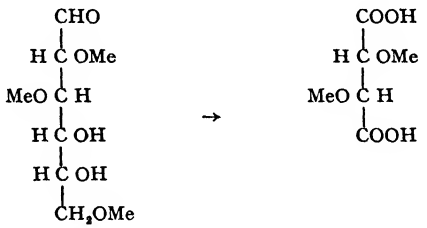
2 : 3 : 4-*Trimethyl glucose* is obtained on hydrolysis of fully methylated gentiobiose, melibiose and raffinose, and $\beta$-glucosan. It can be synthesised by the methylation and subsequent hydrolysis of triphenylmethyl glucose. It is oxidised by nitric acid to xylo-trimethoxyglutaric acid, thus clearly establishing its structure. It is a syrup, but forms a crystalline methyl glucoside which is used for its identification.



The isolation of this trimethyl glucose from the hydrolysis of a methylated disaccharide indicates that the second sugar residue is joined to carbon 6 of the first.

2 : 3 : 6-*Trimethyl Glucose.*—This crystalline sugar is a hydrolysis product of trimethyl cellulose and trimethyl starch, and of fully methylated cellobiose, maltose and lactose. It was obtained from cellulose by Denham and Woodhouse.[8]

Its structure is established as follows: it forms no osazone: therefore position 2 is methylated. On further methylation it gives tetramethylglucopyranose: therefore position 5 is free. Oxidation with nitric acid yields dimethyl-*d*-tartaric acid. Since it is not identical with 2 : 3 : 4 trimethyl glucose it must have the structure



The above formula has been written in the open-chain form, while it is recognised that the crystalline substance has a glucopyranose structure. The position of the methyl groups is established independently of the ring structure. In solution there is no reason why small amounts of glucofuranose forms should not be present in the equilibrium, as is true with glucose itself.

The isolation of 2 : 3 : 6 trimethyl glucose from the hydrolysis products of a methylated polysaccharide indicates that carbons 4 and 5 are those concerned in the linkage to the adjoining sugar residue and the formation of the oxide ring: it does not, however, indicate which carbon has which of these two functions; but it is possible to determine this question unequivocally by two methods. (See page 160.)

Several monomethyl glucoses have been prepared. Helferich[9] has used 3- and 4-monomethyl glucose for the preparation of their methylglucosides in his studies on the specificity of emulsin. Neither are split by the enzyme.

2-*Monomethyl glucose* is prepared by the following series of reactions which establish its constitution.[10]

Glucosemonoacetone → 3 : 5 : 6-tribenzoylglucosemonoacetone → 3 : 5 : 6-tribenzoyl glucose → 3 : 5 : 6-tribenzoylmethyl glucoside → 2-monomethyl-3 : 5 : 6-tribenzoyl-γ-methyl glucoside → 2-monomethyl-γ-methyl glucoside → 2-monomethyl glucose.

Brigl[11] obtained it from the tetrabenzoate of glucose ethylmer-

captal. It is obtained by both methods crystalline m.p. 157°$[\alpha]_D$—66°. It has been incorrectly described as 4-methyl glucose by Pacsu.[12]

3-*Monomethyl glucose* is obtained on hydrolysis of the methylation product of glucose diacetone.[13] It is crystalline. Its constitution follows from that of glucose diacetone.

6-*Monomethyl glucose* was prepared by Helferich and Becker.[14]

The other sugars lend themselves to methylation, just as glucose. The criticism that structural alterations may occur during methylation has always to be met, but so far no instance of this occurring has been noted. Hudson's [15] objections to the Haworth formula for mannose may be set aside, because they are known to be due to unjustifiable use of optical rotation rules which have definite limitations.[16]

METHYL ETHERS OF GLUCOSE.

| | Melting-Point. | Rotation $[\alpha]_D$. |
|---|---|---|
| 2-methyl glucose | 157° | − 66° |
| 3-methyl glucose (1 : 5) | 160° | +107°→68° |
| 4-methyl glucose (1 : 5) | 156° | + 32°→52° |
| 5-methyl glucose (1 : 4) | 143° | +101°→60° |
| 6-methyl glucose | Syrup | + 80°→66° |
| α-2 : 3-dimethyl glucose | 85° | 82°→48° |
| β-2 : 3-dimethyl glucose | 108° | 6°→49° |
| 2 : 3 : 4-trimethyl glucose (1 : 5) | Syrup | + 70° |
| 2 : 3 : 6-trimethyl glucose (1 : 5) | 122° | + 69° |
| 3 : 4 : 6-trimethyl glucose (1 : 4) | Syrup | |
| α-2 : 3 : 4 : 6-tetramethyl glucose (1 : 5) | 103° | +114°→85° |
| β-2 : 3 : 4 : 6-tetramethyl glucose (1 : 5) | 50° | + 73°→85° |
| 2 : 3 : 5 : 6-tetramethyl glucose (1 : 4) | Syrup | − 27° |
| 2 : 3 : 4 : 6-tetramethyl-α-methyl glucoside | 148°-150° | +151° |
| 2 : 3 : 4 : 6-tetramethyl-β-methyl glucoside | 40°-41° | − 17° |
| 2 : 3 : 5 : 6-tetramethyl-α-methyl glucoside | Syrup | +104° |
| 2 : 3 : 5 : 6-tetramethyl-β-methyl glucoside | Syrup | − 64° |
| 2 : 3 : 4 : 5 : 6-pentamethyl glucose | Syrup | − 35° |

## Acetone Derivatives.

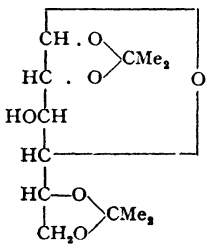
The acetone derivatives of the sugars are formed when the finely divided sugar is shaken in acetone suspension in the presence of a small amount of hydrogen chloride, zinc chloride or other suitable catalyst. Many of them are crystalline. The sugars form mono and diacetone derivatives, and the sugar alcohols give rise to triacetone compounds. It was early recognised [17] that the isopropyl residue of acetone was joined to the *cis* hydroxyl groups of neighbouring carbon atoms. The actual structure of these compounds has been for a long time a subject of controversy (Fischer left their constitution undetermined). Clarity has come about as the result of our

appreciating that the reaction is one in which the liability to form acetone derivatives depends on the spatial proximity of *cis* hydroxyl groups, and that it is this possibility which influences whether the acetone compound has a furanose or a pyranose structure.

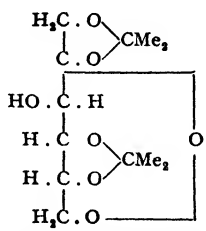
Glucose yields a diacetone derivative,[18] more properly termed di-*iso*propylidene glucose, which only contains one free hydroxyl group. This can be methylated, and after elimination of the acetone residue a crystalline monomethyl glucose is obtained which has proved to have the methyl in the 3-position and to be a derivative of glucopyranose (the full proof is given by Haworth, *Sugars*, Arnold, 1929, pages 47-49).

Controlled hydrolysis converts glucosediacetone into glucosemonoacetone which, like the acetone sugars generally, is stable to alkali and to Fehling's solution. The trimethyl glucose formed from it by methylation and subsequent hydrolysis is capable of forming an osazone, a fact which places the acetone residue on carbons 1 and 2 in the monoacetone derivative.[19] The above trimethyl glucose is converted into tetramethylglucofuranose on further methylation, a fact which both establishes the furanose structure of glucose diacetone and assigns positions $C_5$ and $C_6$ to the second acetone group.

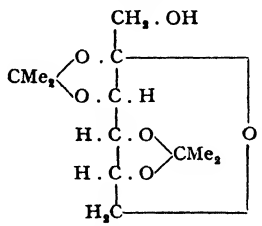
The formula thus is



According to the conditions fructose condenses with acetone to give two crystalline products distinguished as α- and β-fructose diacetones. They have been shown to have the following formulæ by Ohle and Koller,[20] who have discussed the mechanism of their formation:—



α-Di-*iso*propylidenefructose.



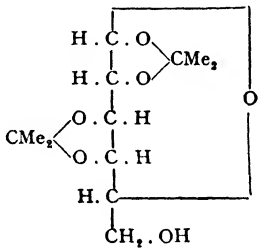
β-Di-*iso*propylidenefructose.

Each of them is stable to alkali and non-reducing and, therefore, has the ketonic group substituted ; it is also stable to oxidation.

α-Fructose diacetone forms a crystalline monomethyl derivative which passes on hydrolysis by acids into a crystalline monomethylfructose [19] which has been proved to be 3 : methylfructopyranose. β-Fructosediacetone [21] can be oxidised by alkaline permanganate to a monobasic acid, indicating that one of the terminal hydroxyls is free. By methylation of this acid the exact position of the acetone groups has been established (Haworth, l.c.).

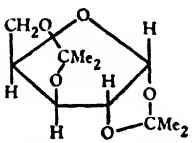
A monoacetone of fructose, viz. 2 : 3-isopropylidenefructofuranose has been made by Zervas.[22]

Galactose forms a diacetone[23] in which the hydroxyl on $C_6$ is free. This is clearly indicated by its conversion into the 6-iodohydrin and reduction of this to *d*-fucose diacetone, from which *d*-fucose is obtained on acid hydrolysis to remove the acetone groups.[24]



Mannose, on the other hand, gives rise to a diacetone in which the reducing group is free. It displays mutarotation and can be readily oxidised to give γ-mannonolactone diacetone whence the proof that it is a mannofuranose.[25] α-methylmannoside (a pyranoside) also gives rise to the same diacetone showing that in the α-glucoside like the sugars the ring alters to accommodate the acetone residue.[26]

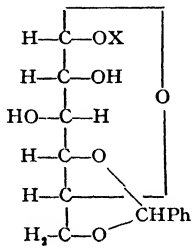
Considerable interest attaches to xylose diacetone in which one of the acetone groups condenses with the hydroxyls on the third and fifth carbons instead of with hydroxyls on contiguous carbons. The formula of xylose as written below indicates the spatial proximity of these two groups to be close : it is apparently equivalent to that of two *cis* hydroxyl groups attached to contiguous carbon atoms.



Diacetone xylose

*l*-Arabinose gives rise to a monoacetone derivative,[27] m.p. 110°, $[\alpha]_D$ + 128·8°, which reduces Fehling's solution, and therefore like mannose has the reducing group free.

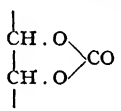
Glucose and the methyl glucosides combine with benzaldehyde forming benzylidene derivatives [31] in which positions $C_4$ and $C_6$ are substituted. Such compounds are useful for further syntheses :—




Similarly, paraldehyde condenses to give 4 : 6 ethylidene glucose.[32]

## The Sugar Carbonates.

The crystalline dicarbonates of the sugars of the type




were first obtained by Haworth and Porter,[28] by the action of carbonyl chloride. They are well defined and easily characterised products, and resemble closely the diacetone derivatives, except that they are less readily hydrolysed by dilute acid. They are assigned structural formulæ similar to those of the diacetones :—

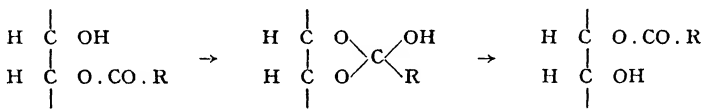
| | M.p. | $[\alpha]_D$. |
|---|---|---|
| Glucose dicarbonate . . | 224° | − 29° in acetone |
| Galactose ,, . . . | 212° | − 86·5° |
| Mannose ,, . . . | 122° | + 26° |
| Fructose ,, . . . | 173° | − 143° |
| Arabinose ,, . . . | 200° | + 61° |

The carbomethoxy derivatives [29] are of the same type as the pentacetyl compounds, each — OH being substituted by — O . CO . OEt. With methyl chloroformate and alkali, however, the sugars yield products which are both carbonates and carbomethoxy derivatives.[30]

## Acyl Derivatives of the Sugars.

The hydroxyl groups of the sugars exert an alcoholic function and can be esterified with acids. The resulting acetates, benzoates, toluene sulphonates, etc., have proved very useful compounds in developing the synthetic side of sugar chemistry. The natural gallotannins belong also to this class of compounds. Those compounds which possess ring structures exist in α- and β-modifications exactly as the sugars themselves and their methylglycosides.

A limitation to the use of acyl derivatives for determination of structure has lately become specially evident in that the wandering of acyl groups to unsubstituted hydroxyls may take place during subsequent operations. This, as Emil Fischer found, is due to the intermediate formation of an ortho acid.



Migration tends to occur from a tertiary to a secondary, and a secondary to a primary alcohol group.

## Glucose Acetates.

*Glucose Pentacetate.*—Five hydroxyl groups in glucose can be substituted to give a pentacetate, the α- or β-pentacetate predominating in the product according to the method employed.

To obtain the α-pentacetate it is necessary to acetylate the glucose rapidly before conversion to the equilibrium mixture of α- and β-glucose, which is accelerated by acid or alkali, can occur. This is achieved by adding anhydrous α-glucose to boiling acetic anhydride containing a small quantity of zinc chloride as catalyst. After the vigorous reaction the product is poured into water, in which the acetate is insoluble, and finally the acetate solidifies. The crude product contains both isomerides: it is purified by crystallisation from alcohol. The α-pentacetate predominates also when glucose is acetylated in pyridine solution at 0°.

To obtain the β-pentacetate, glucose is mixed with acetic anhydride and sodium acetate, and heated for some time at the temperature of the water bath. Under these conditions the change from α- to β-glucose precedes acetylation, and β-glucose pentacetate predominates in the final product, and may be separated by fractional crystallisation.

When heated with acetic anhydride either form is partially converted into the other until equilibrium is attained, when 90 per cent. of the α- and 10 per cent. of the β-form are present.

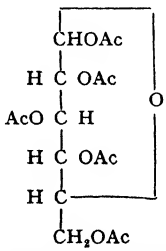
In a similar manner the pentose sugars give rise to tetracetyl derivatives $C_5H_6O(CH_3COO)_4$.

The acetates are insoluble in water, soluble in organic solvents. They do not mutarotate. The acetyl groups are removed by alkalis, most conveniently with ammonia in alcoholic solution.

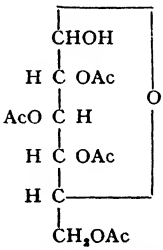
Helferich [42] has found a simplified method of preparing glucosides, applicable to phenolic glucosides only, which consists in fusing the appropriate phenol with pentacetyl glucose and a small amount of acid catalyst, such as zinc chloride or toluenesulphonic acid. The former catalyst apparently favours α-glucoside formation and the latter the production of β-glucosides.

α- and β-methylglucoside on acetylation are converted into tetra-acetates, which on hydrolysis with acids yield the tetracetyl glucoses, unsubstituted on carbon 1 and therefore able to mutarotate.

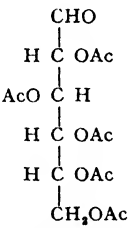
Aldehydoglucose pentacetate has been described on page 27.



Pentacetyl glucose.



Tetracetyl glucose.



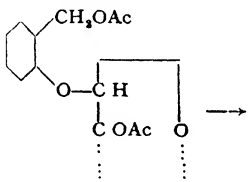
Pentacetyl aldehydo-glucose.

*Glucose Esters of Higher Fatty Acids.*—Compounds obtained by coupling fatty acids with carbohydrates were first prepared by Lapworth and Pearson; their structure has been investigated by Gilchrist.[33] Crystalline penta-palmityl and stearyl glucoses were synthesised by Hess and Messmer.[34] A natural product of this type is crocin, a carotenoid pigment which is the di-gentiobioside of the dicarboxylic acid crocetin $C_{20}H_{24}O_4$.

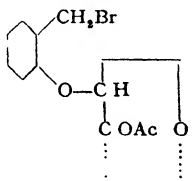
*Acetohalogenoglucoses.*—The acetyl group attached to carbon 1 in glucose pentacetate differs from the other four acetyl groups in reactivity, and is readily replaced by the halogens or by the nitro group. Such groups are very reactive, and these compounds are invaluable for the condensation of the glucoside residue with other compounds, a variety of agents being used for the purpose.

Acetobromoglucose, first prepared by the action of acetyl bromide on glucose,[35] was later obtained from the pentacetates by treatment with liquid dry hydrogen bromide.[36] The most convenient method [37] is by the action of hydrobromic acid in acetic acid on the pentacetate or by simultaneous bromination and acetylation of glucose by a solution of hydrobromic acid in acetic anhydride.

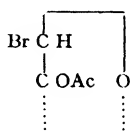
The dextrorotatory acetochloroglucose (+ 166°) and acetobromoglucose (+ 198°) are assigned to the *alpha* series according to Hudson's system of nomenclature, but this does not necessarily express their relation to α- or β-glucose. The conversion of either compound to β-methylglucoside, does not involve a Walden inversion as was once thought.[38] It is apparently the presence of the halogen atom which causes carbon 1 to rotate in the opposite direction to that shown when the methoxy group is attached. Evidence for this view is afforded by the change in rotation from negative to positive when pentacetyl salicin is converted into tetracetyl salicin bromide, the bromine being substituted for the acetyl group of the aglucone.[39]



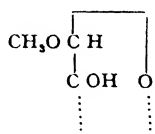
β-Pentacetyl salicin.
$[\alpha]_D$ − 18·5°.



α-Tetracetyl salicin bromide.
$[\alpha]_D$ + 43·3°.



α-Acetobromoglucose



β-Methylglucoside.

α-Acetobromoglucose has the same configuration therefore as β-methylglucoside.

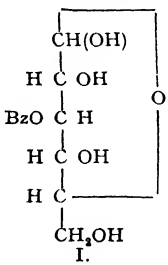
The lævorotatory acetochloroglucose (− 13°) is the β-form : [40] it is prepared under stringent conditions by the action of silver chloride on acetobromoglucose in presence of boiling ether. It readily becomes isomerised during preparation, but, once isolated, it may be crystallised from pure ether. In carbon tetrachloride, benzene and ether, it is relatively stable, but rapidly exhibits mutarotation in chloroform. Owing to the ease with which β-acetochloroglucose isomerises, it yields only the β-glucosides.

The successive action of aluminium chloride and phosphorus pentachloride on an ice-cold solution of tetracetylglucose in chloroform leads almost exclusively to the α-form [41] of acetochloroglucose.

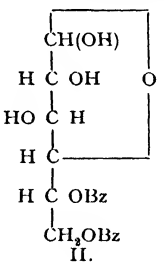
## The Benzoyl Glucoses.

The acetone sugars have proved very useful as a means of introducing other groups into the sugar molecule in known places. The formation of mono-, di- and tribenzoyl glucose may be cited as an example of such a use.[43] Thus when diacetone glucose is benzoylated the benzoyl radical enters at carbon 3: dilute acids remove the two acetone residues, leaving 3-benzoyl glucose I. To obtain 5 : 6-dibenzoyl glucose II. the successive stages are acetylation of diacetone glucose to put an acetyl group at $C_3$; hydrolysis to eliminate one acetone residue, subsequent benzoylation, and finally hydrolysis to remove the remaining acetone residue.[44] The synthesis depends on the relative strength of attachment of acetone, acetyl and benzoyl residues, and it is assumed there is no wandering of the acyl groups.

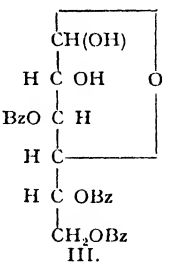
If 3 : 5 : 6-tribenzoylglucose III. is desired, monoacetone glucose is fully benzoylated and the product hydrolysed.[45]



I.

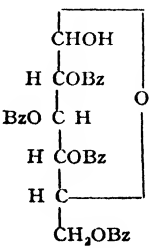


II.



III.

The fully acylated 1 : 2 : 3 : 4 : 6-pentabenzoyl glucopyranose [46] is best obtained by the action of benzoyl chloride and pyridine or quinoline in chloroform solution on glucose. When this is treated in acetic acid solution with hydrogen bromide tetrabenzoylbromoglucose results [47] from which, when the bromine is eliminated, 2 : 3 : 4 : 6-tetrabenzoylglucose is obtained.[43]



The isomeric α- and β-pentabenzoylglucofuranose 1 : 2 : 3 : 5 : 6 derivatives have also been obtained crystalline.[48] 1-Monobenzoylglucose is prbduced on benzoylation of 4 : 6-benzylideneglucose.[49]

BENZOYL GLUCOSES.

| | M.p. | $[\alpha]_D$ |
|---|---|---|
| α-Pentabenzoylglucose 1 : 5 . . . | 187° | + 138° |
| β-Pentabenzoylglucose 1 : 5. . . . | 157° | + 24° |
| α-Pentabenzoylglucose 1 : 4 . . . | 118° | + 79° |
| β-Pentabenzoylglucose 1 : 4 . . . | 146° | − 82° |
| 2 : 3 : 4 : 6-Tetrabenzoylglucose 1 : 5 . . | 119° | + 70·6° |
| 2 : 3 : 5 : 6-Tetrabenzoylglucose 1 : 4 . . | — | + 12° |
| 3 : 5 : 6-Tribenzoylglucose . . . . | 65° | − 75° |
| 2 : 3 : 4-Tribenzoylglucose . . . . | 189° | — |
| 3 : 5-Dibenzoylglucose . . . . | 145° | + 56° → + 66° |
| 3-Monobenzoylglucose . . . . | 95° | + 47° |
| 1-Monobenzoylglucose . . . . | 193° | − 26·8° |

## Triphenylmethylglucose : *p*-Toluenesulphonylglucose.

The hydroxyl on carbon 6 displays a number of special reactions. Thus triphenylmethyl chloride acts on glucose, galactose and their glycosides, the triphenylmethyl group entering position 6 preferentially [50] though according to several workers it is also capable of entering other as yet unknown positions. These ethers are hydrolysed both by alkali hydroxide and by hydrochloric acid, so that they afford a means of obtaining glucose derivatives with a free hydroxyl on $C_6$ and from this a 6-monomethylglucose.

*p*-Toluenesulphonyl residues also enter position 6, which can be identified by conversion of the sugar into the corresponding glycoside, substituting the vacant hydroxyl groups by *p*-toluenesulphonyl residues and heating the product with sodium iodide in acetone. Under these conditions the substituent in position 6 is replaced quantitatively by iodine.[10], [51]

By further operations iodine can be replaced by nitrate and this by hydroxyl. The properties are strictly confined to the 6-position, and the various compounds crystallise with ease.

## The Gallotannins.

A number of sugar esters of acids of the aromatic series are plant products; they range in complexity from the simple monobenzoyl glucose vaciniin, to the complex Chinese gallotannin, in which ten gallic acid residues are combined with one molecule of glucose.

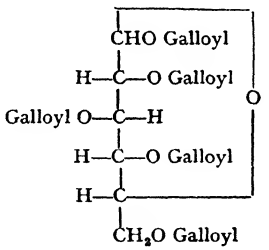
Vaciniin is found in cranberries and is 6-monobenzoylglucose.[52] An isomer exists in the glycoside populin, found in poplar bark, where the aglycone is salicyl alcohol. A dibenzoyl gluco-xylose was found by Power and Salway [53] in *Daviesia latifolia.*

A number of the anthocyanin glycosides contain various acids substituting their sugar components, for example, *p*-hydroxy cinnamic acid in monardaein, gentianin and violanin and *p*-hydroxybenzoic acid in delphinin. Such compounds are widely distributed.[54]

The simplest galloyl sugar is 1-galloyl glucose or glucogallin found by Gilson [55] as a constituent of Chinese rhubarb, and synthesised by Fischer and Bergmann.[56] Mention may be made here of the analogous structure possessed by crocin, the digentiobioside of crocetin.

Hamameli tannin contains a digalloyl derivative of the sugar hamamelose (page 139). A digalloyl glucose is among the hydrolysis products of the tanning material, chebulinic acid.[57]

Turkish gallotannin is obtained from the gall nuts of the oak, *Quercus infectoria,* from Asia Minor. It yields on hydrolysis five molecules of gallic acid to one of glucose. The simplest expression of the formula is as a pentagalloyl glucose analogous to pentabenzoylglucose.




Actually this picture is idealised. The tannin can be fractionated into portions of different rotatory power and is clearly not homogeneous. The hydrolysate contains small amounts of ellagic acid, a condensation product of two molecules of gallic acid, and there is evidence for the presence of digallic acid (*m*-galloyl gallic acid) residues. It may be said that on the average one out of five hydroxyls is unsubstituted, three are esterified with gallic acid, and one with digallic or ellagic acids.

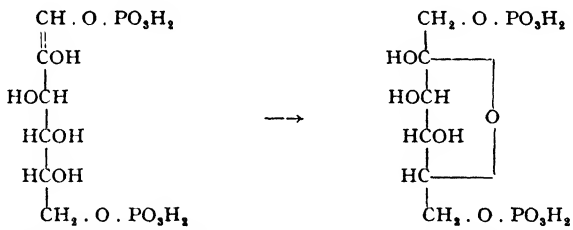
Chinese gallotannin from the leaf galls of *Rhus semialata* of Eastern Asia is more complicated, yielding about nine molecules of gallic acid to one of glucose. The simplest expression is that the tannin contains four *m*-digalloyl residues and one galloyl residue. The

substance is not homogeneous and is probably a mixture of octa-, nona- and decagalloylglucoses with the digalloyl residues occupying several alternative positions. The presence of trigalloyl residues is doubtful, though not excluded.[58]

Fischer and Freudenberg [59] synthesised pentagalloylglucose, and Fischer and Bergmann [60] penta-*m*-digalloyl glucose. These synthetic compounds showed themselves to resemble very closely the natural tannins in all physical and chemical properties, and constitute as near a proof as can be obtained of the structure of substances which are not homogeneous.

## Phosphoric Esters.

The first stage in the fermentation of glucose by zymase is the formation of hexose diphosphate, $C_6H_{10}O_4(H_2PO_4)_2$. Glucose, mannose and fructose give rise to the same hexose phosphate: when this is hydrolysed fructose is obtained. This points to the phosphate as arising from the enolic form of the three hexoses: it is formulated as derived from fructofuranose (see Harden, *Alcoholic Fermentation*, 1932, in this series) :—




1 : 6-diphosphoric ester of the enolic form.

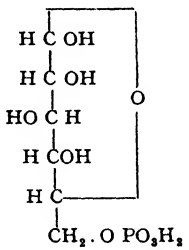
Hexose 1 : 6-diphosphoric ester.

Three different phosphates have been isolated in the form of salts from the fermentation reaction, viz. the diphosphate,[61] a monophosphate, usually known as the Robison ester [62] and a trehalose monophosphate [63] which occurs only in quantity in the products formed by dried yeast.

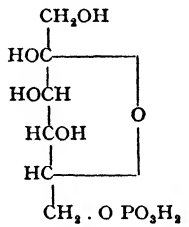
A second different monophosphate described as a fructose compound has been obtained by Neuberg [64] by the partial hydrolysis of the diphosphate. What is probably the same fructose phosphate,[65] obtained by partly hydrolysing sucrose phosphate with dilute hydrochloric acid, is stated to be readily fermented and to reduce Fehling's solution. Apparently in this the hexose residue is in a condition of extreme instability. The free acid has $[\alpha]_D + 1{\cdot}5°$.

The Robison ester is a mixture: it consists mainly of an aldose ester together with some quantity of a ketose ester. Their separation is accomplished by converting the barium salt into the brucine salt and recrystallising from 20 per cent. alcohol.[66] The aldose ester has $[\alpha]_D + 25°$ and is glucopyranose-6-phosphoric acid. On oxidation with bromine it yields a phosphogluconic acid which the enzyme phosphatase converts into *d*-gluconic acid. It yields glucose on hydrolysis by acid to which it is very resistant.[67]

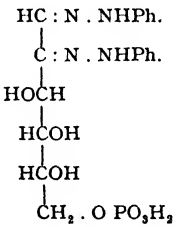
The ketose ester is regarded as identical with Neuberg's ester, and is considered to be fructofuranose-6-phosphoric ester formed by the removal of the readily hydrolysable phosphoric group on $C_1$ of the diphosphate.




Glucopyranose - 6 - phosphoric ester.




Fructofuranose - 6 - phosphoric ester.




Monophospho-phenylosazone.

All three phosphates give rise to the same osazone.

The aldose ester on methylation yields two series of methylhexoside monophosphates which probably correspond with the pyranose and furanose forms of the compound, indicating also that the hydroxyl groups on $C_5$ and $C_4$ are both unsubstituted.

The hexosephosphates are hydrolysed when boiled with acid, but the different esters liberate phosphoric acid at varying rates. The hexosephosphates are also hydrolysed by phosphatases which are widely distributed (Harden, l.c., p. 67). The diphosphate is hydrolysed by the enzyme in stages. (The diphosphoric acid has $[\alpha]_D + 3{\cdot}4°$.) When $\alpha$- and $\beta$-methyl hexoside diphosphoric acids are submitted to the action of phosphatase, $\alpha$- and $\beta$-methyl fructofuranosides are formed.[68]

The trehalose ester has $[\alpha]_D + 185°$. Phosphatase readily removes the phosphoric acid group leaving trehalose, but hydrolysis by acid preferentially forms glucopyranose-6-monophosphate and glucose, as the disaccharide linkage is weaker than that between the phosphoric acid group and the carbohydrate.

There are probably other natural phosphoric esters, for example one indicated in very small quantity by Robison and Morgan.[69]

The synthetic phosphoric esters have been prepared by the action of phosphorus oxychloride on the carbohydrates in presence of calcium carbonate or hydroxide. Neuberg[70] thus describes monophosphates of glucose and galactose: as they only reduce Fehling's solution after hydrolysis the acid residue is considered to be attached to carbon 1. Neither is fermented by yeast.

Glucose-3-monophosphoric ester[71] is formed by the action of phosphorus oxychloride on diacetone glucose in which the only free hydroxyl group is on carbon 3. It has $[\alpha]_D + 39{\cdot}5°$ and is fermented by zymase. The similar ester prepared from monoacetoneglucose is regarded as the 6-ester and is identical with Robison's monoester.

There are a number of other phosphoric esters reported which are not yet very definitely characterised.

Pyrophosphate[72] is not only a constituent of muscle but it is also widely distributed in nature in bacteria, yeast, pea seedlings, in fact in all cells which can utilise carbohydrates.[73] Boyland[74] has shown that it forms about one-fourth of the total phosphorus in living yeast. Both when administered orally[75] and when added to fermenting zymin (Boyland) it is rapidly hydrolysed to orthophosphate. The distribution of phosphorus compounds in fresh brewer's yeast (as mg. of phosphorus per gram of yeast) is given as follows:—

| | | | |
|---|---|---|---|
| Total phosphorus . | 3·25 | | |
| Orthophosphate . | 1·36 | Hexosediphosphate . | 0·38 |
| Pyrophosphate . | 0·68 | Hexosemonophosphate | 0·72 |
| Organic phosphorus | 1·17 | Nucleic acid . . | 0·07 |

In connection with carbohydrate phosphates, those of starch must not be overlooked. Samec[75] has isolated hydrolysis products of high phosphate content approximating to a monophosphate of a disaccharide. Posternak[76] has shown that this biose monophosphate on hydrolysis yields glucose-6-phosphoric ester, identical with that from yeast.

The sugar phosphates are of greatest interest as constituents of the nucleotides. According to Levene[77] the phosphoric acid is attached to the terminal carbon atom $C_5$ of the ribose in inosinic acid and muscle adenylic acid, but to a different carbon atom in yeast adenylic acid. In xanthylic and guanylic acids position $C_5$ in the sugar is free, and the phosphoric acid is attached at $C_3$ (or perhaps $C_2$). (For a full account of the structure of the nucleotides, see Levene and Bass, *The Nucleic Acids*, New York, 1931.)

It will be noted that there is no record of a phosphoric acid residue being attached to group 4 in any of the sugars, such as is necessary to explain the possible conversion of glucose to galactose or of xylose to ribose by a Walden inversion which has been suggested by Robinson on theoretical grounds.

## REFERENCES TO CHAPTER VIII.—THE METHYL GLUCOSES.


1. Purdie and Irvine, J.C.S., 1903, 1021; J.C.S., 1904, 1049.
2. Haworth, J.C.S., 1915, 8.
3. Denham and Woodhouse, J.C.S., 1914, 2357.
4. Purdie and Bridgett, J.C.S., 1903, 1037.
5. Irvine, Fyfe and Hogg, J.C.S., 1915, 524. Micheel and Hess, Ann., 1926, **450**, 21.
6. Levene and Meyer, J.B.C., 1927, **74**, 701.
7. Levene and Meyer, J.B.C., 1926, **69**, 175.
8. Denham and Woodhouse, J.C.S., 244.
9. Helferich and Lang, J. Pr. Chem., 1932, **132**, 321.
10. Oldham and Rutherford, J.A.C.S., 1932, 1086.
11. Brigl and Schinle, Ber., 1930, **63**, 2884.
12. Pacsu, Ber., 1925, **58**, 1455. Levene, Meyer and Raymond, J.B.C., 1931, **91**, 497.
13. Irvine and Scott, J.C.S., 1913, 564.
14. Helferich and Becker, Ann., 1924, **440**, 13.
15. Hudson, J.A.C.S., 1930, 1680, 1707.
16. Haworth, Hirst and Streight, J.C.S., 1931, 1349. Haworth, Hirst and Plant, J.C.S., 1931, 1354.


## ACETONE DERIVATIVES.


17. Fischer, Ber., 1895, **28**, 1145.
18. Irvine and Scott, J.C.S., 1913, 564.
19. Anderson, Charlton and Haworth, J.C.S., 1929, 1329.
20. Ohle and Koller, Ber., 1927, **60**, 1168.
21. Ohle, Koller and Behrend, Ber., 1925, **58**, 2577.
22. Zervas, Ber., 1933, **66**, 1698.
23. Ohle and Behrend, Ber., 1925, **58**, 2585.
24. Freudenberg and Raschig, Ber., 1927, **60**, 1633.
25. Goodyear and Haworth, J.C.S., 1927, 3136.
26. Levene and Meyer, J.B.C., 1928, **78**, 363.
27. Ohle and Behrend, Ber., 1927, **60**, 810.
28. Haworth and Porter, J.C.S., 1930, 151.
29. Zemplen and Laslo, Ber., 1915, **48**, 921.
30. Allpress and Haworth, J.C.S., 1924, 1223.
31. Zervas, Ber., 1931, **64**, 2289.
32. Helferich and Appel, Ber., 1931, **64**, 1841.


## ACYL DERIVATIVES.


33. Gilchrist, Brit. Ass. Rep., 1922, 357.
34. Hess and Messmer, Ber., 1921, **54**, 499.
35. Königs and Knorr, Ber., 1901, **34**, 962.
36. Fischer and Armstrong, Ber., 1901, **34**, 2885.

37. Fischer, Ber., 1916, **49**, 584.
38. Hudson, Dixième Conference de l'Union Internationale de Chimie, Liége, 1930, p. 64.
39. Kunz, J.A.C.S., 1926, 262.
40. Fischer, Ber., 1911, **44**, 1898.
41. Schlubach, Ber., 1926, **59**, 840. Schlubach, Stadler and Wolf, Ber., 1928, **61**, 287.
42. Helferich and Schmitz-Hillebrecht, Ber., 1933, **66**, 378.
43. Fischer and Noth, Ber., 1918, **51**, 321.
44. Ohle, Biochem. Z., 1922, **131**, 611.
45. Fischer, Ber., 1915, **48**, 266; Ber., 1916, **49**, 88, 209.
46. Fischer and Freudenberg, Ber., 1912, **45**, 2725.
47. Fischer and Helferich, Ann., 1911, **383**, 88.
48. Schlubach and Huntenberg, Ber., 1927, **60**, 1487. Levene and Meyer J.B.C., 1928, **76**, 513.
49. Zervas, Ber., 1931, **64**, 2289.
50. Helferich and Becker, Ann., 1924, **440**, 1. Helferich, Moog and Junger, Ber., 1925, **58**, 872.
51. Freudenberg and Raschig, Ber., 1927, **60**, 1633.

## THE TANNINS.

52. Ohle, Ber., 1924, **57**, 403; Ber., 1925, **58**, 2593.
53. Power and Salway, J.C.S., 1914, 767, 1062.
54. Robinson and Robinson, Biochem. J., 1931, **25**, 1687; Biochem. J., 1932, **26**, 1647.
55. Gilson, Bull. Acad. med. Belg., 1927 (4), **16**, 827.
56. Fischer and Bergmann, Ber., 1918, **51**, 1804.
57. Freudenberg and Frank, Ann., 1927, **452**, 305.
58. Freudenberg, *Tannin, Cellulose, Lignin*, Berlin, 1933.
59. Fischer and Freudenberg, Ber., 1912, **45**, 915.
60. Fischer and Bergmann, Ber., 1918, **51**, 1760.

## PHOSPHORIC ESTERS.

61. Harden and Young, Proc. Roy. Soc., 1908, **80 B**, 299.
62. Harden and Robison, Proc. Chem. Soc., 1914, **30**, 16. Robison, Biochem. J., 1922, **16**, 809.
63. Robison and Morgan, Biochem. J., 1928, **22**, 1270; 1930, **24**, 119.
64. Neuberg, Biochem. Z., 1918, **88**, 432.
65. Neuberg, Biochem. Z., 1911, **36**, 5.
66. Robison and King, Biochem. J., 1931, **25**, 323.
67. Levene and Raymond, J.B.C., 1930, **89**, 479; J.B.C., 1931, **92**, 757.
68. Morgan and Robison, Biochem. J., 1928, **22**, 1270.
69. Robison and Morgan, Biochem. J., 1930, **24**, 119.
70. Neuberg, Biochem. Z., 1910, **23**, 515; **26**, 514; 1911, 5.
71. Levene and Raymond, J.B.C., 1930, **89**, 479. Josephson and Proffe, Ann., 1930, **481**, 91.
72. Lohmann, Biochem. Z., 1928, **202**, 466.
73. Lohmann, Biochem. Z., 1928, **203**, 164.
74. Boyland, Biochem. J., 1930, **24**, 350.
75. Samec, Kolloidchem. Beiheft, 1931, **33**, 449.
76. Posternak, Compt. rend., 1934, **198**, 506.
77. Levene and Jacobs, Ber., 1908, **41**, 2703; 1909, **42**, 335, 1198; 1911, **44**, 746. Levene and Simms, J.B.C., 1925, **65**, 31. Levene and Mori, J.B.C., 1929, **81**, 215.

## CHAPTER IX.

### THE AMINO HEXOSES.

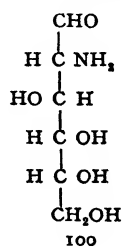
Two of these are known to occur naturally, chitosamine usually termed glucosamine, which is 2-aminoglucose, and chondrosamine, which is 2-aminogalactose. Other amino hexoses have been prepared synthetically.

*Chitosamine or glucosamine = 2-aminoglucose.*—Glucosamine is of interest as having been the first well-defined carbohydrate derivative to be obtained from animals.[1] It is obtained by hydrolysis of the nitrogen containing polysaccharide chitin, which forms part of the skeletal substance of insects and crustacea. Chitin has been identified in the skeletal remains of Coleoptera from the upper middle Eocene, of age twenty-five million years.[2] Irvine [3] concluded that the chitins from various invertebrate animals were identical.

Glucosamine is best prepared from lobster shells by boiling in concentrated hydrochloric acid. The shell consists of calcium carbonate, a small amount of protein material and chitin, which is broken down to glucosamine and acetic acid. Glucosamine can be obtained from its hydrochloride by decomposing it with diethylamine or sodium methoxide.

Chitin is also an important skeletal element in the fungi. E. Winterstein [4] has shown that it does not occur in higher plants. According to Khouvine,[5] it is impossible to distinguish between animal and vegetable chitin by total nitrogen or by X-ray analysis: their chemical identity has been shown by Zechmeister and Toth.[6]

It was for a long time uncertain whether chitosamine was 2-aminoglucose, as suggested by Irvine,[7] or 2-aminomannose as once seemed possible from Levene's stereochemical researches on the derivatives of amino sugars. Levene [8] has finally shown how all the data can be reconciled with the acceptance of chitosamine as 2-aminoglucose.



Glucosamine hydrochloride exists in two isomeric forms which mutarotate, the α-form having $[\alpha]_D$ changing from + 100° to + 72·5° and the β-form $[\alpha]_D$ increasing from + 25° to + 72·6°. On this basis the molecular rotation difference 2A is 16,160, the corresponding constant for glucose being 16,900, whereas that for mannose is 9200, confirming a relationship to glucose rather than mannose.

Glucosamine reduces Fehling's solution; gives with hydrogen cyanide an amino glucoheptonic acid; is oxidised to an amino hexonic acid; forms pentacetyl and pentabenzoyl derivatives; and also an oxime, semicarbazone and phenyl hydrazone; it gives glucose phenyl osazone when heated with phenyl hydrazine, losing the amino group. The phenylisocyanate derivative is characteristic.

Irvine and Hynd [10] were able to transform glucosamine into glucose or alternatively into mannose. Nitrous acid converts glucosamine into an anhydro sugar $C_6H_{10}O_5$ called chitose.[11] Its constitution does not seem to have been finally settled.

The aminohexoses are synthesised from the pentoses [12] by the following series of operations. By means of methylalcoholic ammonia the imine (1-aminopentose) is formed, which is condensed with hydrocyanic acid and the product hydrolysed with hydrochloric acid at 0°. A mixture of the two epimeric hexosaminic acids is obtained in this manner, and on reduction these yield the corresponding aminohexoses. The epimeric acids can be converted into one another by heating with pyridine.

All the 2-amino *d*-hexonic acids (*d*-hexosaminic) have been prepared by Levene and his co-workers, and their configurational relationships to the hexonic acids established. These may be summarised :—

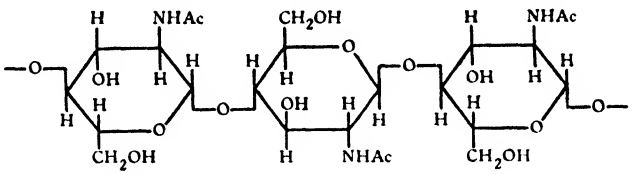
| Hexosaminic Acid. | $[\alpha]_D$ in 2·5 per cent. HCl. | Related Hexonic Acid. |
|---|---|---|
| Chitosaminic ((−) *d*-arabinohexosaminic) | − 15·0° | Gluconic |
| Epichitosaminic ((+) *d*-arabinohexosaminic) | + 10·0° | Mannonic. |
| Chondrosaminic ((−) *d*-lyxohexosaminic) | − 17·0° | Galactonic |
| Epichondrosaminic ((+) *d*-lyxohexosaminic) | + 8·0° | Talonic |
| (−) *d*-xylohexosaminic | − 11·0° | Gulonic |
| (+) *d*-xylohexosaminic | + 14·0° | Idonic |
| (−) *d*-ribohexosaminic | − 26·0° | Allonic |
| (+) *d*-ribohexosaminic | + 12·5° | Altronic |

The more recent work on the structure of chitin indicates that it is built up in a very similar manner to cellulose. This similarity of

structure is in accord with its similarity of function as the skeletal substance in fungi and in crustacea and insects.

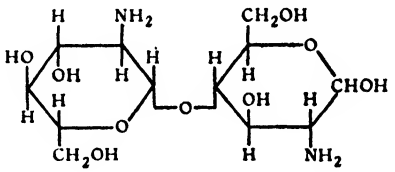
Chitin is only soluble in concentrated acids and is extremely resistant to hydrolysis. In concentrated hydrochloric acid it has a characteristic initial rotation of $-14{\cdot}7°$. Chitin on hydrolysis with concentrated hydrochloric acid yields one molecule of acetic acid to one molecule of glucosamine. Chitin is a polymer of anhydro acetyl glucosamine.

Meyer and Mark [13] suggested that chitin was built up of N-acetyl glucosamine units in $\beta$-glucosidic linkages exactly as in cellulose :—



According to Karrer [14] an enzyme from the intestine of the vineyard snail is able to split chitin which has been dissolved in hydrochloric acid and reprecipitated, to give a 50 per cent. yield of N-acetyl glucosamine. Chitin from *Boletus edulis* yields 80 per cent. N-acetyl glucosamine.[15]

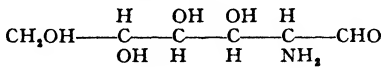
Bergmann [16] has been able to obtain by the acetolysis of chitin with acetic acid in sulphuric acid the octacetate of a disaccharide chitobiose to which he assigns the structure



The analogy with the production of cellobiose from cellulose makes the Meyer and Mark formula very probable. The number of units in the molecule is not yet established. Zechmeister [17] has reported that a partially degraded chitin or chitodextrin is hydrolysed by emulsin which at first sight affords strong support for the $\beta$-glycosidic union of the acetyl-glucosamine units. It is surprising in view of such information as we possess on the specificity of the $\beta$-glucosidase of emulsin that it should be able to hydrolyse chitodextrin, particularly in view of Bergmann's finding that chitobiose could not be hydrolysed by either emulsin or maltase.

According to Helferich,[19] although the phenol glycoside of N-acetyl glucosamine is split by emulsin, this is not due to the β-glucosidase enzyme, nor to the α-mannosidase component. Zechmeister and Grassmann agree that the chitin-splitting by emulsin is not due to the simple β-glucosidase component.

*Chondrosamine = 2-Aminogalactose.*



was first described as glucosamine, but the osazone from it was subsequently identified as galactosazone. It has been investigated by Levene and La Forge.[19]

Progress in the difficult subject of the mucins and mucoids is largely due to Levene [20] who has summarised his researches in a monograph of this series. Mucins of different origin contain conjugated sulphuric acids, either chondroitin or mucoitin, which differ in their amino sugar constituents.

Cartilage (nasal septa), tendons, aorta, sclera, contain chondroitin sulphuric acid ; this is a tetrasaccharide consisting of two glucuronic acid and two chondrosamine units, in which the amino groups are acetylated and the primary alcohol groups esterified with sulphuric acid. On hydrolysis the sulphuric and acetic acids are split off and the molecule ruptured between the two glucuronic acid molecules forming chondrosin. Levene has obtained mucoitin sulphuric acid from a number of mucins and mucoids. This differs from chondroitin since on hydrolysis it yields a disaccharide composed of glucuronic acid and glucosamine which is termed mucosin $C_{12}H_{21}O_{11}N$. Glucosamine is also found combined with mannose as a constituent of various proteins.

## Other Amino Sugars.

*Iso*glucosamine was obtained by Fischer [21] by reducing phenylglucosazone with zinc dust and acetic acid. It is 1-aminofructose.

$$CH_2(OH) . (CHOH)_3 . CO . CH_2 . NH_2$$

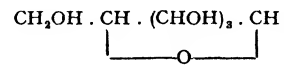
It forms salts, and bears the same relation to fructose as glucosamine does to glucose. The reaction is a general one, and can be extended to other osazones.

The glucose aldehyde ammonia prepared by Ling and Nanji [22] by the action of dry ammonia on glucose suspended in methyl alcohol, yields on reduction glucamine $CH_2OH . (CHOH)_4 . CH_2NH_2$ which

is also obtained in the reduction of the oxime of glucose, a more general method for the preparation of these compounds.[23]

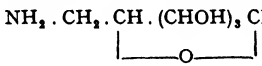
On recrystallisation from methyl alcohol, glucose aldehyde ammonia loses a molecule of water forming glucosimine, first obtained by Lobry de Bruyn.[24]

Similar compounds have been obtained from other sugars.[25] A number of different ways of formulating these compounds are possible, of which one is



3-aminoglucose,[26] or epiglucosamine, is obtained by the action of alcoholic ammonia at 175° on diacetone-3-toluenesulphonylglucose.

6-aminoglucose [27] was obtained by Fischer and Zach by the following series of operations: β-pentacetylglucose, when treated with anhydrous liquid hydrogen bromide, forms dibromo-triacetylglucose which reacts with methyl alcohol to give triacetyl β-methylglucoside 6-bromohydrin. This is converted by ammonia at the ordinary temperature into amino β-methylglucoside from which the amino sugar is obtained on hydrolysis. Ohle and Vargha [28] found an easier way by the addition of ammonia to 5 : 6-anhydroglucose.



The corresponding 6-aminogalactose [29] is obtained by the action of ammonia on a 6-toluenesulphonyl diacetonegalactose.

These compounds yield an osazone which still contains the amino group.

## REFERENCES TO CHAPTER IX.—AMINO HEXOSES.


1. Ledderhose, Z. Physiol. Chem., 1878, **2**, 213.
2. Abderhalden and Heyns, Biochem. Z., 1933, **259**, 320.
3. Irvine, J.C.S., 1909, 564.
4. Winterstein, Z. Physiol. Chem., 1893, **10**, 521; Ber., 1895, **28**, 167.
5. Khouvine, Compt. rend., 1932, **195**, 396.
6. Zechmeister and Toth, Z. Physiol. Chem., 1934, **223**, 53.
7. Irvine, J.C.S., 1912, 1128.
8. Levene, Chemical Reviews, 1925, **2**, 179.
9. Steudel, Z. Physiol. Chem., 1902, **34**, 353.
10. Irvine and Hynd, J.C.S., 1914, 698.
11. Fischer and Andreae, Ber., 1903, **36**, 2587.
12. Fischer and Leuchs, Ber., 1903, **36**, 24.
13. Meyer and Mark, Ber., 1928, **61**, 1936.
14. Karrer and Hoffman, Helv. Chim. Acta, 1929, **12**, 616.

15. KARRER AND FRANCOIS, Helv. Chim. Acta, 1929, **12**, 986.
16. BERGMANN, ZERVAS AND SILBERKWEIT, Ber., 1931, **64**, 2436.
17. ZECHMEISTER, GRASSMANN, TOTH AND BENDER, Ber., 1932, **65**, 1706.
18. HELFERICH, Z. Physiol. Chem., 1933, **221**, 252.
19. LEVENE AND LA FORGE, J.B.C., 1914, **18**, 123. LEVENE, J.B.C., 1915, **20**, 443.
20. LEVENE, *The Hexosamines and Mucoproteins.*
21. FISCHER, Ber., 1886, **19**, 1921.
22. LING AND NANJI, J.C.S., 1922, 1682.
23. MAQUENNE AND ROUX, Compt. rend., 1901, **132**, 980.
24. LOBRY DE BRUYN, Rec. trav. chim., 1894, **12**, 286; 1895, **14**, 134.
25. FISCHER AND LEUCHS, Ber., 1902, **35**, 3790. IRVINE, THOMPSON AND GARRETT, J.C.S., 1913, 241.
26. FREUDENBERG, BURKHART AND BRAUN, Ber., 1926, **59**, 714.
27. FISCHER AND ZACH, Ber., 1911, **44**, 132.
28. OHLE AND VARGHA, Ber., 1928, **61**, 1207.
29. FREUDENBERG AND DOSER, Ber., 1925, **58**, 294.

# CHAPTER X.

## GLUCAL, GLUCOSEEN, GLUCOSONE, GLUCOSAN.

*Glucal:* $HO.CH_2.CH.CH(OH).CH(OH).CH{=}CH$ (with O bridging the CH and CH=CH)

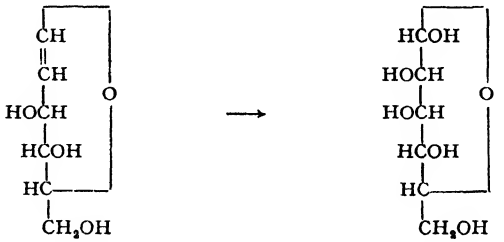
WHEN acetobromoglucose is reduced by zinc dust and acetic acid an unsaturated substance is produced, termed triacetylglucal by Fischer.[1] Glucal [2] is prepared from it by hydrolysis with methyl-alcoholic ammonia, when it is obtained crystalline, m.p. 60°, $[\alpha]_D - 7{\cdot}2°$.

Ozone breaks down glucal to *d*-arabinose, proving the position of the double bond; [3] hydrogenation converts it into the stable hydro-glucal; sulphuric acid into desoxyglucose.

The crystalline dihalogeno- addition products of glucal are mixtures of isomerides as two carbon atoms become asymmetric in their formation. The halogen attached to the terminal carbon is easily exchanged for methoxyl and isomeric methylglucoside-2-bromohydrins are formed, which differ in the configuration of the bromine atom on carbon 2.

Whereas one form is very resistant to hot strong hydrochloric acid but easily attacked by ammonia, the other shows exactly the reverse behaviour; ammonia forms epiglucosamine. On reduction of either form of methylglucoside bromohydrin with sodium amalgam $\beta$-2-desoxymethylglucoside is formed.

When oxidised with perbenzoic acid glucal is almost quantitatively converted into mannose.[2]




This reaction affords both a method of converting glucose into mannose and an example of an asymmetric synthesis, one epimer

being produced almost exclusively. On the other hand, trimethylglucal gives mainly, if not entirely, trimethylglucose,[4] and 3-methylglucal yields 3-methylglucose [5] which again are asymmetric syntheses in the opposite sense.

Triacetylglucal, however, when treated with perbenzoic acid and water, gives a mixture of both glucose and mannose derivatives. Further, the 4-galactosidohexose obtained from lactal is a mixture of two sugars.[6]

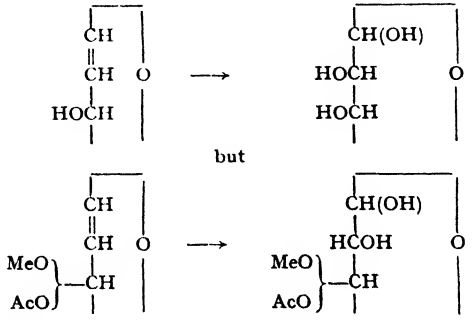
The nature of the oxide ring in glucal cannot be deduced with certainty from its formation from a glucopyranose compound in view of the ease with which acetyl groups can migrate. The fact that 4-glucosidoglucose yields cellobial and 6-glucosidoglucose yields gentiobial both containing glucal, and the properties of 3-methylglucal are against the presence of 1 : 4, 1 : 6 or 1 : 3 rings. The 1 : 5 ring is confirmed by the conversion of trimethylglucal into tetramethylglucopyranose.[4]

The conversion of glucal into α-methylmannoside with a pyranose structure and the formation of triacetylglucal from acetobromomannose is also evidence for the normal pyranose structure of mannose.

Similar glycals are obtained from the other sugars, both monosaccharides and disaccharides; their reaction with perbenzoic acid has been studied by Levene and Tipson [7] with a view to the synthesis of some of the rarer sugars.

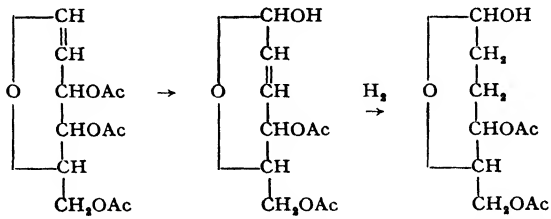
Since the configuration difference between epimeric sugars is destroyed by the introduction of the double bond between $C_1$ and $C_2$, *d*-glucal is identical with *d*-mannal, and *d*-xylal with *d*-lyxal, etc.[8]

Galactal (talal) with perbenzoic acid yields mainly talose and a little galactose.[9] It is considered that the group attached to $C_3$, either OH, OMe, OAc, has the power of directing whether the hydroxy group enters at $C_2$ predominatingly cis or trans:




Rhamnal gives rise exclusively to rhamnose.

*Pseudoglucal.*—When glucal triacetate is boiled with water the double bond is shifted down the chain and the diacetate of *pseudo*-glucal is obtained.[9]



Glucal triacetate. *Pseudo*glucal. Bisdesoxyglucose.

Pseudoglucal is reduced to a bisdesoxyglucose.

*Isoglucal.*—When diacetyl pseudoglucal is hydrolysed with baryta an *iso*glucal is obtained.

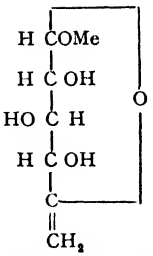
Isoglucal. Protoglucal.

The structure has been established by Bergmann, Zervas and Engler,[10] mainly by analogy with the more tractable isolactal prepared from lactose.

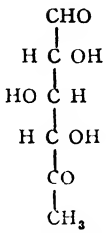
*Protoglucal.*—A by-product in the production of *iso*glucal above is another substance $C_6H_8O_3$ which Bergmann calls *proto*glucal and is probably identical with the substance Fischer [11] first gave the name glucal to, because of its aldehydic properties.

When the tetracetates of glucose 6-halogenohydrins, or methyl-glucoside 6-halogenohydrin are treated in pyridine with silver salts the hydrogen halide is abstracted forming an unsaturated glucoseen.[12]

Free glucoseen 5 : 6 is not known, for when $\beta$-methyl-glucoseenide is hydrolysed by acids the product changes over to the 5-keto methyl-pentose called isorhamnonose by Helferich and Himmen.[13]



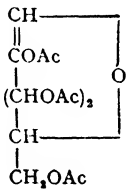
Methyl-glucoseenide.



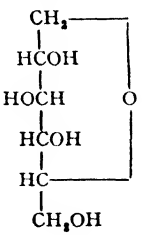
Isorhamnonose.

The isomeric fuconose is obtainable in a similar manner from diacetone galactoseen.

*Glucoseen* 1 : 2.—A second glucoseen is obtained by the action of aliphatic secondary amines, e.g. diethylamine, on acetobromoglucose, when hydrogen bromide is abstracted.[14] The compound is only known as its tetracetate, an alternative name for which is tetracetyl oxyglucal. The reaction is a general one for sugars.

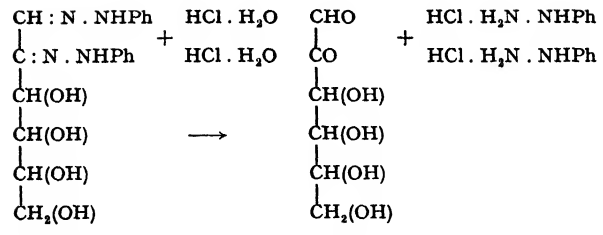


This 1 : 2 glucoseen tetracetate has enabled the synthesis of the natural styracitol $C_6H_{12}O_5$ to be effected; it is formed on reduction and subsequent hydrolysis. proving it to be 1 : 5-anhydro-*d*-sorbitol.[15]



Glucoseen 1 : 2 tetracetate can be converted into crystalline acetates of glucosone,[16] which are converted in pyridine solution into kojic acid with great readiness.

*Glucosone.*—Fuming hydrochloric acid acts on the phenylosazone in the same manner as it does on the hydrazone of glucose, eliminating both hydrazine groups to form an osone.[18]




Phenylosazone. Osone.

Glucose, mannose and fructose, which form the same phenylosazone, likewise form the same osone. The osones are colourless syrups and have not yet been crystallised ; they act as strong reducing agents, and combine directly with phenylhydrazine or with disubstituted phenylhydrazines forming osazones. The osones combine also with *o*-phenylene diamine. They are not fermentable. On reduction glucosone is converted into fructose. This is the only method available of regenerating a sugar from the phenylosazone. When the sugar originally used was an aldose the corresponding ketose results. The method is of great historical interest, as by its aid Fischer [19] established the nature of the synthetical α-acrose. Morrell and Crofts [20] obtained the same osone by oxidation of glucose, fructose or mannose with hydrogen peroxide and a ferrous iron salt. It is also formed by the action of light on glucose in presence of a catalyst. According to Dixon and Harrison,[21] glucosone can be obtained by the oxidation of fructose with selenious acid. Walker [22] has found that glucosone is produced from glucose in cultures of *Aspergillus oryzae* of the *flavus* type grown in presence of toluene.

The osones of the disaccharides [23] may be obtained from their respective osazones, which are soluble in boiling water, by means of benzaldehyde which removes both phenylhydrazine residues. These osones are similar to glucosone in properties : they are hydrolysed by enzymes in the same way as the parent disaccharides.

### *The 2-Desoxy Sugars.*

These were at first prepared synthetically from glucal : now that they have also been identified as natural products, largely owing to the work of Levene, a very much greater interest attaches to them.

The natural desoxy sugars are the crystalline digitoxose (page 139) obtained by Kiliani [24] in 1895 from a digitalis glycoside, which he later recognised as a desoxymethylpentose,

$$CH_3 . CH(OH) . CH(OH) . CH(OH) . CH_2 . CHO,$$

and the thyminose or *d*-2-desoxyribose,

$$CH_2(OH) . CH(OH) . CH(OH) . CH_2 . CHO,$$

discovered in thymonucleic acid by Levene.[25]

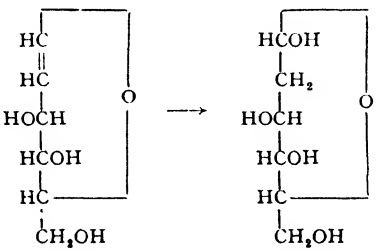
Cymarose, the sugar of strophanthin, is also a desoxy sugar being represented provisionally as

$$CH_3 . CH(OH) . CH(OH) . CH(OCH_3) . CH_2 . CHO:$$

and the sarmentose [26] from sarmentocymarin is an isomer.

The desoxy sugars are unstable and pass on standing into a green coloured tar; they do not form osazones, and are characterised by giving a blue colour when 4 or 5 drops of sulphuric acid containing ferrous iron is added to their solution in glacial acetic acid.[27]

Desoxyglucose (2-glucodesose) is prepared from glucal by treatment in the cold with dilute sulphuric acid.[28] The method is a general



one, and by it *l*-2-desoxyribose (= 2-desoxyarabinose) and 2-desoxy-*d*-xylose (= 2-desoxy-lyxose) have been prepared; apart from the optical isomerides there are no other desoxypentoses possible.

Crystals of either α- or β-2-desoxyglucose can be obtained at will by concentrating the aqueous solutions and seeding. In aqueous solution either form gives a final rotation $[\alpha]_D + 46{\cdot}6°$, mutarotation proceeding very rapidly, but in pyridine the rotation of the β-form changes from + 15° to + 90°, the α-form having $[\alpha]_D + 90°$. On reduction 2-desoxysorbitol is obtained, and on oxidation with bromine 2-desoxygluconic acid is formed.

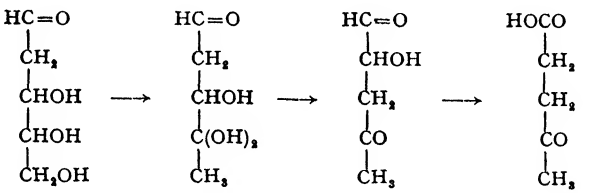
The desoxyglucoses reduce Fehling's solution, and react as aldehydes with fuchsin sulphurous acid solution.

They have a far greater reactivity than the simple sugars: thus they are very readily converted into the desoxyglycosides. In fact the velocities of formation and hydrolysis of these are approximately of the same order as those of the furanose glucosides, so that it has been questioned whether they are not likewise of this structure. The evidence at present is in favour of a 1 : 5 ring. It is based on the

preparation of a methylated lactone by Levene and Mikeska,[29] and on the formation of a desoxyglycoside with similar properties from 4-glucosidoglucal, i.e. cellobial,[30] in which the $C_4$ position is occupied. Desoxycellobiose is similar in properties to desoxyglucose.

α-Methyldesoxyglucoside has $[\alpha]_D + 137{\cdot}8°$, whereas the β-isomeride has $[\alpha]_D - 48{\cdot}2°$; the difference in the specific rotations being approximately the same as that between the methylglucosides. Neither glycoside is affected by yeast extract nor the β-glycoside by emulsin. Phenyl-α-2-desoxyglucoside is split by emulsin, by the same enzyme component which hydrolyses α-mannosides.

The desoxypentoses are readily converted by the action of mineral acids into lævulinic acid, the reaction probably taking place in the following stages according to Levene and Mori,[31] by dehydration and hydration.

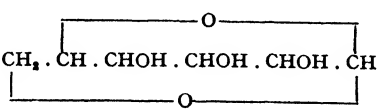


In consequence of this change, the sugar of the nucleosides was for a long time held to be lævulinic acid.

## Sugar Anhydrides.

### *Glucosan.*

When cellulose, starch or certain natural glucosides are dry distilled under reduced pressure, a crystalline anhydroglucose known as lævo-glucosan is produced.[32] Irvine and Oldham [33] have converted this glucosan into trimethylglucosan and trimethylglucose and established it as β-glucosan with the structure



Pictet [34] assigned the above structure to lævoglucosan, and deduced from it the space formula of β-glucose as having the OH groups on carbons 1 and 2 on opposite sides of the carbon chain (see page 24). β-Glucosan has no structural relationship to cellulose, starch, or the glucosides, but is undoubtedly formed by the dehydration of β-glucose

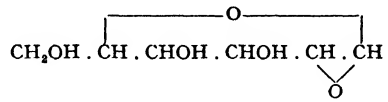
when quickly heated under reduced pressure: it can be distilled unchanged, the product having m.p. 178° to 180°, $[\alpha]_D$ — 66·2°.

According to Karrer [35] β-glucosan is obtained quantitatively on warming tetracetylglucoside trimethylammonium bromide with alkali.

β-Glucosan yields 2 : 3 : 4-trimethyl-β-glucosan, which is a simple 1 : 6 anhydride and has none of the properties of a methylated saccharide, though the corresponding trimethylglucose is that obtained from methylated starch, glycogen and cellulose.[36]

Pictet,[37] following Gelis, has shown that by heating α-glucose at 150° to 155° under a pressure of 15 mm. α-glucosan is obtained $[\alpha]_D$ + 69·4°, m.p. 108° to 109°. It is very deliquescent, reduces Fehling's solution, and when boiled with water gives glucose again.

It is considered by him to possess an ethylene oxide structure

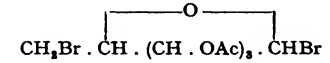


The preparation of a "reducing" trimethylglucosan by Cramer and Cox [38] confirms this formula; α-glucosan yields a 3 : 4 : 6-trimethyl derivative when methylated with methyl sulphate.

α-Glucosan dissolves in concentrated hydrochloric acid to form α-glucosylchloride in which the chlorine atom is mobile, rendering the compound useful for the synthesis of glucosides and disaccharides.

The glucosans are polymerised on heating, α-glucosan giving a diglucosan at 15 mm. and a tetraglucosan at ordinary pressure. These polymerides have lost their aldehydic nature. Better results are obtained using zinc chloride to effect polymerisation. Arabinose,[39] rhamnose,[40] fructose,[41] galactose,[42] and other sugars give similar products when heated.

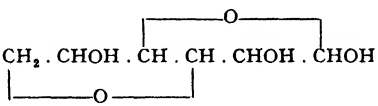
3 : 6-*Anhydroglucose.*—When the action of anhydrous hydrogen bromide on glucose pentacetate is prolonged, dibromo-triacetyl glucose



is obtained.[43] One of the bromine atoms can be displaced by methoxyl with the formation of triacetyl methylglucoside bromohydrin. This compound has served as the starting-point for a number of syntheses, among them the preparation of 6-aminoglucose (page 104). When it is heated with barium hydroxide, hydrogen bromide is eliminated and anhydro methylglucoside, $C_7H_{12}O_5$, is formed; this on

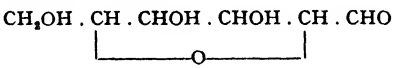
cautious hydrolysis by acids yields anhydroglucose, a well-characterised crystalline substance. It forms a phenylhydrazone and phenylosazone, both containing one molecule of water less than the corresponding glucose compounds. Strong acids convert anhydroglucose back into glucose: reduction forms anhydrosorbitol and oxidation yields anhydrogluconic acid.[44] Unlike glucose it has the property of restoring the colour of Schiff's reagent.

Its most probable constitution is that of a 3 : 6-anhydroglucose containing a furanose ring,



That the second bromine atom in dibromo-triacetyl glucose is in the 6-position is proved by its reduction to the methyl pentose, isorhamnose.[45] This is supported by the preparation of the anhydro-sugar from glucose monoacetone.[46] The di-*p*-toluenesulphonyl derivative of the glucose monoacetone when treated with alkali is converted into a crystalline mono-*p*-toluenesulphonyl derivative of the anhydro-sugar which on further treatment with alkali gives a crystalline anhydroglucose monoacetone. Dilute acids eliminate the acetone residue leaving the free anhydro-sugar.

2 : 5-*Anhydroglucose*, or chitose, is obtained by the action of nitrous acid on glucosamine. The corresponding derivative of mannosamine is called epichitose. The crystalline sugar may exist either in the free aldehyde form or as a furanose.



2 : 4-*Anhydroglucose* is obtained from the action of concentrated hydrochloric acid on β-glucosan after removing other products by fermentation.[47] Pringsheim [48] had formulated it as 1 : 6-glucose.

## REFERENCES TO CHAPTER X.


1. Fischer, Ber., 1914, **47**, 196.
2. Bergmann and Schotte, Ber., 1921, **54**, 440.
3. Fischer, Bergmann and Schotte, Ber., 1920, **53**, 509.
4. Hirst and Woolvin, J.C.S., 1931, 1131.
5. Levene and Raymond, J.B.C., 1930, **88**, 513.
6. Walters and Hudson, J.A.C.S., 1930, 3472.
7. Levene and Tipson, J.B.C., 1931, **93**, 631.
8. Gehrke and Obst, Ber., 1931, **64**, 1724.

9. BERGMANN AND FREUDENBERG, Ber., 1931, **64**, 158. BERGMANN AND BREUERS, Ann., 1923, **434**, 79; Ann., 1929, **470**, 51.
10. BERGMANN, ZERVAS AND ENGLER, Ann., 1933, **508**, 25.
11. FISCHER AND ZACH, Sitzungsber. preuss. Akad. Wiss., Berlin, 1913, **16**, 311.
12. HELFERICH AND HIMMEN, Ber., 1928, **61**, 1825.
13. HELFERICH AND HIMMEN, Ber., 1929, **62**, 2136.
14. MAURER, Ber., 1929, **62**, 332.
15. ZERVAS, Ber., 1930, **63**, 1689.
16. MAURER AND PETSCH, Ber., 1931, **64**, 2011.
18. FISCHER, Ber., 1888, **21**, 2632; Ber., 1889, **22**, 88.
19. FISCHER AND TAFEL, Ber., 1890, **23**, 98.
20. MORRELL AND CROFTS, J.C.S., 1899, 788; J.C.S., 1900, 1221; J.C.S., 1902, 668.
21. DIXON AND HARRISON, Biochem. J., 1932, **26**, 1954.
22. WALKER, Nature, 1932, **130**, 582.
23. FISCHER AND ARMSTRONG, Ber., 1902, **35**, 3141.

## DESOXY SUGARS.

24. KILIANI, Ber., 1905, **38**, 4040; Arch. Pharm., 1916, **254**, 261.
25. LEVENE AND LONDON, J.B.C., 1929, **81**, 711; J.B.C., 1929, **83**, 793. LEVENE AND MORI, J.B.C., 1929, **83**, 803; J.B.C., 1930, **85**, 785.
26. JACOBS AND BIGELOW, J.B.C., 1932, **96**, 335.
27. KILIANI, Arch. Pharm., 1913, **251**, 567.
28. BERGMANN, SCHOTTE AND LECHINSKY, Ber., 1922, **55**, 158.
29. LEVENE AND MIKESKA, J.B.C., 1930, **88**, 791.
30. BERGMANN AND BREUERS, Ann., 1929, **470**, 38.
31. LEVENE AND MORI, J.B.C., 1929, **83**, 108.

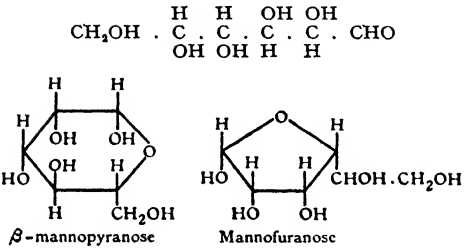
## SUGAR ANHYDRIDES.

32. PICTET AND SARASIN, Helv. Chim. Acta, 1918, **1**, 87.
33. IRVINE AND OLDHAM, J.C.S., 1921, 1744.
34. PICTET, Helv. Chim. Acta, 1920, **3**, 649.
35. KARRER AND SMIRNOFF, Helv. Chim. Acta, 1921, **4**, 817.
36. MICHEEL AND HESS, Ber., 1927, **60**, 1898.
37. GELIS, Compt. rend., 1860, **51**, 331. PICTET AND CASTAN, Helv. Chim. Acta, 1922, **5**, 884.
38. CRAMER AND COX, Helv. Chim. Acta., 1922, **5**, 884.
39. VOGEL, Helv. Chim. Acta, 1928, **11**, 1210.
40. VOGEL, Helv. Chim. Acta, 1928, **11**, 442.
41. PICTET AND REILLY, 1921, **4**, 613.
42. PICTET AND VOGEL, 1928, **11**, 209.
43. FISCHER AND ARMSTRONG, Ber., 1902, **35**, 833.
44. FISCHER AND ZACH, Ber., 1912, **45**, 456, 2068.
45. FISCHER AND ZACH, Ber., 1912, **45**, 3761.
46. OHLE, VARGHA AND ERLBACH, Ber., 1928, **61**, 1211. OHLE AND EULER, Ber., 1930, **63**, 1796. FREUDENBERG, TOEPFFER AND ANDERSON, Ber., 1928, **61**, 1750.
47. REICHEL AND ERDÖS, Ber., 1932, **65**, 1618.
48. PRINGSHEIM AND KOLODNY, Ber., 1926, **59**, 1135; 2241.

# CHAPTER XI.

## THE ALDO AND KETO HEXOSES.

### Mannose.




In spite of careful searches *d*-mannose has never been found [1] to occur free in nature, although it is widely distributed in the polysaccharides of the hemicellulose type known as mannans or mannosans which are converted into mannose when hydrolysed by acids. Mannans are found particularly in the wood of conifers and in the hard skins and woody parts of seeds. A convenient source for the preparation of mannose is the vegetable ivory nut, which is the endosperm of the seed of the Tagua palm, *Phytelephas macrocarpa*, and is used in button manufacture. A method of obtaining as much as 40 per cent. of the weight of the vegetable ivory in the form of pure crystalline mannose was worked out in detail by Hudson and improved by Clark.[2] The meal is hydrolysed by 75 per cent. sulphuric acid, the acid removed by means of barium carbonate and, after decolorising, the solution is evaporated to a thick syrup and mixed with an equal volume of glacial acetic acid. This method improves on the original practice of Fischer and Hirschberger of isolating the phenylhydrazone and regenerating the sugar from this derivative; it renders the sugar easily available.

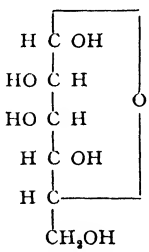
Considerable quantities of mannose have been obtained from white spruce cellulose; another source is the officinal Salep mucilage which is composed solely of mannose and is obtained from tubers

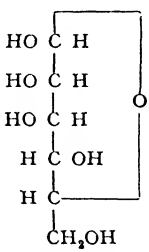
of *Orchidaceae*. Mannose occurs in seaweeds [3] along with *l*-fucose, and is a major constituent of yeast gum. The substance known as Konjakmannan, obtained from the roots of a Japanese *Arac*, and a favourite article of food in the far East, is a polysaccharide which gives two molecules of mannose to one of glucose on hydrolysis. According to Nishida and Hashima [4] it can be degraded to a crystalline trisaccharide containing two molecules of mannose and one of glucose.

More recently mannose has been found as a product of hydrolysis of animal substances. Thus it has been obtained from egg albumin and ovomucoid,[5] from horse and ox blood serum globulins.[6] In these compounds it is present as a trisaccharide composed of glucosamine and two molecules of mannose, in which the union of two sugar residues is through the amino group. Mannose has also been identified in tubercle bacilli.[7]

Mannose forms rhombic crystals and has initially a sweet taste followed immediately by a distinctly bitter one. This bitterness is quite characteristic and of interest, in view of the pure sweetness of the closely related isomerides glucose and fructose, the latter being the sweetest sugar known.

Ordinary mannose, which, according to Hudson's nomenclature, is the $\beta$-form, has $[\alpha]_D - 17°$.


α-Mannose.


β-Mannose.

$\alpha$-Mannose first obtained by Levene has $[\alpha]_D + 30°$. The value for the equilibrium mixture is $+ 14{\cdot}6°$. Assuming that other modifications are present only in negligible amounts, an equilibrated aqueous solution contains approximately 33 per cent. of $\alpha$- and 67 per cent. of $\beta$-mannose. To obtain $\beta$-mannose crystals the solution in boiling 96 per cent. alcohol is inoculated with pure crystals under precautions to exclude all access of $\alpha$-mannose.[8] The $\alpha$-mannose is obtained by allowing a mannose solution in alcohol to evaporate in a dish in the open air and inoculating. It also crystallises from aqueous ammonia or glacial acetic acid.[9]

β-Mannose has a higher molecular refractivity, and is less soluble in water than α-mannose. It has, in short, all the properties which in the glucose series are characteristic of α-glucose.

Mannose is the true aldehyde of mannitol, and may be obtained from it by oxidation, or converted into it by reduction. It was first prepared by Fischer and Hirschberger [10] in this manner, and only subsequently identified as a natural product. It is very similar to *d*-glucose in its general properties, exhibits mutarotation, and forms the same phenylosazone as glucose and fructose. Mannose forms a characteristic, sparingly soluble, phenylhydrazone which enables it to be very easily identified. This hydrazone is precipitated within a few minutes when phenylhydrazine is added to a solution of mannose.

Tetramethylmannose is obtained from its epimer, tetramethylglucose, on treatment with dilute alkali in the same way as the unsubstituted sugar from glucose.[11]

By addition of hydrogen cyanide to mannose and hydrolysis of the resulting nitrile two isomeric mannoheptonic acids should in theory be produced, but in fact only one is formed. Such a process is an asymmetric synthesis. If the heptonolactone is reduced to the heptose and the synthesis carried up to the octose and the nonose, the synthesis continues to be asymmetric. The creation of a new asymmetric centre makes two isomers possible, but since they are not related as image and mirror image they will have different energy contents, and are therefore produced at different rates and are obtained in unequal quantities. Only with mannose, however, is one isomer obtained to the practical exclusion of the other.

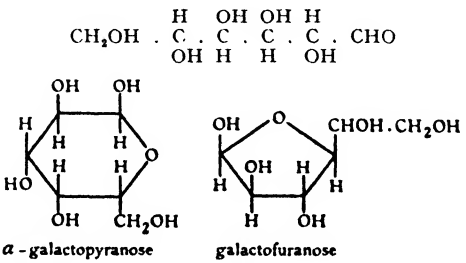
Asymmetric syntheses are the rule in biological processes, and it is seen how the presence of one asymmetric compound can condition the asymmetry of compounds synthesised through its intervention.

The fact that the rotations of certain derivatives of mannose do not conform to Hudson's rules of isorotation has given rise to the suggestion that normal mannose cannot contain a 1 : 5-oxygen ring. The work of Isbell [12] and also of Haworth has established, however, that similar derivatives of 4-glucosido-mannose are likewise exceptional. The molecular rotations of 4-glucosido-α- and β-mannose parallel those of α- and β-mannose. As position $C_4$ is occupied by a glucose residue in these compounds, a 1 : 4-oxygen ring formula for mannose is not possible in them. Hudson who, as already mentioned, contends that a change of ring form may and does occur during the methylation of sugars, has ascribed to α-mannose and to α-methylmannosides a different ring structure from that of β-mannose and

the α- and β-glucopyranoses and their glucosides. Hudson's theory postulates an unknown form of α-mannose which he calculates should have $[\alpha]_D + 77°$ and which, according to his scheme, should be a constituent of the disaccharide 4-glucosido-α-mannose instead of the usual form of α-mannose $[\alpha]_D + 30°$.

It has, however, been shown by Haworth [13] and his co-workers that when 4-β-glucosido-α-methylmannoside is hydrolysed by means of emulsin the normal pyranoside variety of the methylmannoside is obtained. Again methylation methods show that both the α- and β-forms of methylmannoside obtained from mannose have the same pyranose ring structure.[14] The limitations of the isorotation rules of Hudson and the reason for their non-applicability for derivatives of mannose and also of lyxose and rhamnose is discussed in Chapter IV. These three sugars which have the hydroxyl groups on carbons 2 and 3 in *cis* positions, are grouped together by similarities in a number of properties and contrasted with other sugars. Either the α- or β-forms of lyxose, rhamnose and mannose will have three hydroxyl groups on contiguous carbon atoms on the same side of the chain, a configuration which finds expression in their solubilities. It is found, for example, that the proportion of the *trans* or α-form of these sugars tends to be greater in alcohol than in water, a state of affairs which is reversed with glucose and galactose in which sugars the hydroxyls on $C_2$ and $C_3$ are in the *trans* position.

**Galactose.**




*d*-Galactose occurs as a constituent of milk-sugar,[15] also of raffinose and stachyose; it occurs in hemicelluloses as a polymeric galactan or galactosan, but no such polymer yields wholly galactose on hydrolysis; it is a component of pectin where it is in association with arabinose and galacturonic acid; and it is found in gums and mucilages. Galactosides are found among the flavone and anthocyanin glycosides and in the saponins, but are not common.

Lippmann [16] records the appearance of galactose as a crystalline efflorescence, resembling hoar-frost, on ivy berries following a sharp frost, the first after a late dry autumn: free galactose has not otherwise been found in nature. Both isomeric forms of galactose occur naturally. Anderson [17] has prepared *l*-galactose in 11 per cent. yield from flax seed mucilage. *dl*-Galactose was recognised by Winterstein [18] in Chagual gum and by Oshima and Tollens [19] in Japanese Nori.

Under abnormal conditions galactose appears in the sugar-beet in combination with sucrose as the trisaccharide, raffinose: the quantity of raffinose is increased by disturbances of growth, such as those occasioned by sudden frost.

Galactose is the sugar of the brain whence it was isolated, and described under the name cerebrose, by Thudichum. It is a constituent of the cerebrosides phrenosin, also called cerebron, kerasin and nervon, which occur in nerve and brain tissue in considerable amounts.

These compounds on hydrolysis furnish galactose, the base sphingosine and a fatty acid. The sugar is in glycosidic union with the sphingosine, according to Pryde and Humphreys [20] as galactopyranose. Cerebron yields cerebronic acid $C_{24}H_{48}O_3$, kerasin, lignoceric acid $C_{24}H_{48}O_2$, and nervon, nervonic acid $C_{24}H_{46}O_2$: the constitution of these, of sphingosine and the structure of the cerebrosides has lately been established by Klenk.[21] It would appear that nature has made provision during the early stages of brain development for an adequate supply of galactose in the form of milk sugar. Since the transformation of glucose to galactose takes place only in the mammary gland, the importance of feeding lactose and not sucrose during infancy becomes evident.

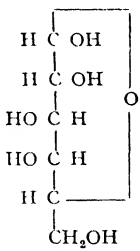
Under normal conditions the blood transports glucose to the mammary glands, where, in the regular course of lactation, it is converted into the disaccharide, milk sugar, and excreted in the milk. Removal of the mammary glands results in an accumulation of glucose in the blood, from which it passes to the urine. Galactose is not found in the urine. Injection of glucose causes lactosuria, when the mammary glands are in full activity, but produces glucosuria when the glands are less active. Nothing is known as to the mechanism by which the mammary glands are able to transform glucose into lactose.

Galactose is easily made by hydrolysis of milk sugar with sulphuric acid: it is crystallised from dilute methyl alcohol and recrystallised from acetic acid. It is less soluble than glucose. It resembles glucose in properties; characteristic is the formation of mucic acid on oxidation with nitric acid, and this may be used for its identification. On

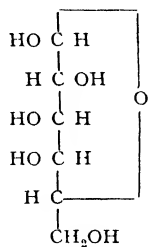
reduction with sodium amalgam the corresponding alcohol, dulcitol, is formed; this is found naturally. By the action of alkalis it is transformed into *d*-talose and *d*-tagatose. It is fermented by some yeasts, but not by all those which ferment glucose; a fact which has been taken as indicating that a special galacto-zymase is required for the fermentation.

It is more apparent with galactose than with glucose that the aqueous solution contains further isomers than the two α- and β-pyranose forms. The mutarotation data,[22] on the simplest possible interpretation indicate that at least three and possibly four different modifications are present in solution: there is no evidence for the presence in appreciable quantities of any aldehyde form.

α-Galactose $[\alpha]_D + 145°$ is obtained in quantitative yield by evaporating an aqueous solution which is in contact with crystalline nuclei of α-galactose. β-Galactose $[\alpha]_D + 54°$ is similarly obtained by evaporating a previously heated solution at room temperature *in vacuo*, taking precautions to prevent extraneous nuclei from reaching the solution.



α-Galactose



β-Galactose.

In pyridine solution Schlubach and Prochownick[23] found the specific rotation of galactose to fall rapidly with rise of temperature, more rapidly than could be accounted for by shift of the equilibrium between α- and β-forms, and attributed this to the appearance of a furanose form in solution. In confirmation of this they found they could obtain from such a solution 20 per cent. of galactofuranose pentacetates.

Galactose may be transformed into *d*-fucose by the following series of operations which effect the reduction of the primary alcohol group:—[24]

galactose → diacetonegalactose → 6-*p*-toluene sulphonyl derivative
→ 6-iodohydrin → diacetone-*d*-fucose.

When galactose is subjected to methyl galactoside formation in the cold it yields a strongly lævorotatory $\gamma$-form from which 2 : 3 : 5 : 6-tetramethyl methylgalactoside $[\alpha]_D$ — 45·2° is prepared. The rotations of the normal $\alpha$- and $\beta$-tetramethyl methylgalactosides are + 143·4° and + 30·7° respectively. On hydrolysis 2 : 3 : 5 : 6-tetramethyl $\gamma$-galactose is formed $[\alpha]_D$ — 21·2° which contrasts with + 109° for the normal 2 : 3 : 4 : 6-tetramethyl galactose. $\beta$-Ethyl galactofuranoside has been prepared in crystalline form, m.p. 86°, $[\alpha]_D$ — 97·2°.[25]

Micheel and Suckfüll [26] have been able to synthesise two isomeric pentacetates of galactose containing a 1 : 6-oxide ring, the first instance of a sugar derivative having this structure, though Helferich and Sparmberg [27] have shown that simple 6-hydroxy aldehydes can form 1 : 6-rings.

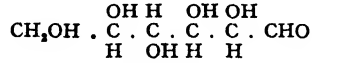
Micheel [28] has converted the pentacetylgalactose 1 : 6 into the corresponding $\alpha$-methylgalactoside-1 : 6.

A complete series of pentacetates of galactose is known, and it is of interest to compare their physical properties.

| Ring Type | M.p. | $[\alpha]_D$. | $[M]_D$. | Diff. 2A. |
|---|---|---|---|---|
| 1 : 4 $\alpha$ | 87° | 61° | 23,800 | 40,200 |
| $\beta$ | 98° | — 42° | — 16,400 | |
| 1 : 5 $\alpha$ | 96° | 107° | 41,700 | 32,730 |
| $\beta$ | 142° | 23° | 8,970 | |
| 1 : 6 $\alpha$ | 128° | — 11° | — 4,300 | 26,100 |
| $\beta$ | 101° | — 78° | — 30,400 | |
| aldehyde | 120° | — 25° | — | — |

The effect of different ring structures on the contribution of the potentially aldehydic carbon to the total molecular rotation is seen.

## Gulose.



*l*-Gulose.

*l*-Gulose was first made as a syrup by Fischer and Piloty [29] by the reduction of *l*-gulonolactone, prepared by the reduction of saccharic acid or glucuronic acid. *d*-Gulose was made by Fischer and Stahel [30] by converting *d*-xylose into the nitrile by addition of hydrogen cyanide, followed by reduction of the *d*-gulonolactone formed

on hydrolysis of the nitrile. In studies of the relation between configuration and optical rotatory power, derivatives of *d*-gulose have been investigated by Isbell.[31] Starting from the crystalline calcium chloride compound of *d*-gulose which consists principally of α-gulose, a mixture of methylgulosides is obtained by the usual methods: these can also be separated by means of their crystalline calcium chloride addition compounds. Their tetracetates have also been prepared crystalline.

The optical values for the gulosides are

| | $[\alpha]_D$. |
|---|---|
| α-methyl-*d*-guloside | + 109·4° |
| β-methyl-*d*-guloside | — 83·3°. |

## Fructose.

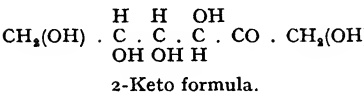
*d*-fructose or lævulose, discovered by Dubrunfaut in 1847, occurs together with glucose in the juices of fruits, in honey, etc., the mixture being often termed fruit sugar or invert sugar. Combined with glucose it occurs as cane sugar, raffinose, gentianose, etc. The polysaccharide inulin which serves as a reserve material like starch and is found in the rhizomes and nodules of *Compositae* and related groups, for example in chicory and the Jerusalem artichoke, yields fructose alone when hydrolysed.

A large number of ill-defined lævulosans, yielding fructose on hydrolysis, and of the same structure as inulin though of smaller molecular weight, have been described, and are evidently of wide distribution. Hibbert has investigated the polysaccharide lævan made up of fructose, which is produced by the action of *B. subtilis* or *B. mesentericus* on sucrose or raffinose.

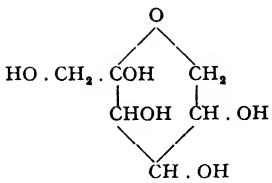
To prepare fructose from invert sugar or hydrolysed inulin it is best to form the crystalline calcium lævulosate and decompose this with carbon dioxide. Inulin is easily hydrolysed under pressure by water containing carbon dioxide.

As a commercial sugar fructose offers great possibilities on account of its sweetness; it is twice as sweet as glucose: desiccated tubers of the artichoke are considered an economic source for its manufacture on the large scale.

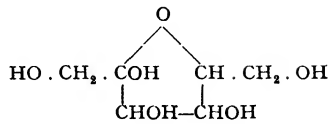
Fructose is a ketohexose having the following alternative constitutions:—



2-Keto formula.



Fructopyranose.
Amylene 2 : 6-oxide.



Fructofuranose.
Butylene 2 : 5-oxide.

Fructose and its derivatives crystallise much less easily than glucose. On solution it exhibits mutarotation due to the conversion of the $\beta$-form to the stereoisomeride, but further changes occur, and in aqueous solution an equilibrated mixture of several isomerides is probably present. This explains the change produced in the specific rotatory power by changes of temperature. The rotatory power becomes less negative as the temperature is increased, and at 87·3° it is equal and opposite to that of glucose, so that a solution of invert sugar at this temperature should be optically inactive.

The work of Irvine and his school has afforded evidence that fructose is much more prone than is glucose to react in the $\gamma$-form. A fresh solution of fructose in water is quite stable to permanganate and contains the pyranose form, but if the solution is made acid, kept for an hour and then neutralised, permanganate is decolorised within a few minutes, showing that a reactive $\gamma$-form of fructose is present. Fructose thus reacts either as—

(1) $\alpha$- and $\beta$-fructopyranose.

Ordinary fructose, the $\beta$-modification, forms rhombic crystals, $[\alpha]_D$ — 133·5°, increasing to — 92° in solution. The calculated rotation of the $\alpha$-form is — 21°.

Fructopyranose is not oxidised by permanganate, does not combine readily with acetone, and forms stable fructosides and acetyl derivatives, e.g. a pentacetate and tetracetyl-$\beta$-methylfructoside, also a crystalline tetramethylfructose $[\alpha]_D$ — 125°.

The $\beta$-pentacetate gives rise to two isomeric acetochlorofructoses when acted on by phosphorus pentachloride: the $\beta$-form, m.p. 108°, $[\alpha]_D$ + 45° and an $\alpha$-isomeride, m.p. 83°, $[\alpha]_D$ — 160·9°, the latter arising when aluminium chloride is present.

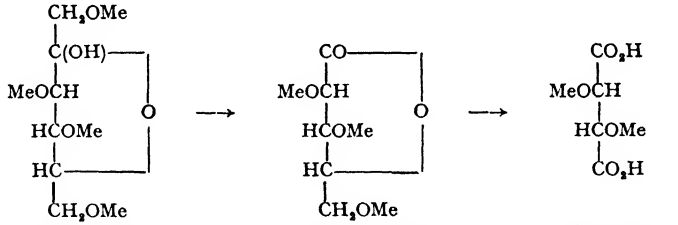
$\alpha$-Methylfructoside has been proved to have a pyranose structure through its characteristic derivative crystalline tetramethylfructose,

which on degradation leads to the same *d*-arabotrimethoxyglutaric acid as is obtained from α-methylmannoside.[32]

(2) A more active compound, conveniently called γ-fructose, or in Germany, *h*-fructose,[33] probably fructofuranose, and likewise capable of existing in interconvertible α- and β-modifications having lower specific rotations than normal fructose. The derivatives of this form are highly reactive; it combines readily with acetone and reduces permanganate. The γ-fructosides are very easily hydrolysed by acids and resemble sucrose, which is a derivative of fructofuranose. The γ-tetramethyl derivative is a liquid having $[\alpha]_D + 29{\cdot}3°$.

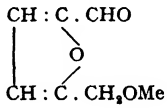
γ-Fructose has been formulated in turn as an ethylene oxide by Irvine and Robertson,[34] as an amylene oxide by Haworth and Linnell [35] and as a butylene oxide by Haworth and Hirst, whereas Hudson,[36] who considers that the oxygen bridges shift during methylation, formulates it with a propylene oxide ring. Hudson ascribes a butylene oxide ring to normal fructose.

The evidence for the furanose structure of tetramethyl-γ-fructose is based on its degradation through trimethyl fructuronic acid to trimethyl-γ-arabonolactone which has been further degraded to dimethoxysuccinic acid.[37]




Tetramethyl-γ-fructose. 2 : 3 : 5-Trimethyl-arabonolactone. Dimethoxy-succinic acid.

Confirmatory evidence of this structure for tetramethylfructose is afforded by its conversion into ω-methoxy methylfurfural,




identified through its oxime and semicarbazone, as well as through the corresponding acid. The transformation takes place with great ease in contact with either dilute mineral acid or with acetic acid and sodium acetate.

Fructose reacts very rapidly with methyl alcohol containing 0·5

per cent. hydrogen chloride—in 40 minutes at 20° about 85 per cent. of a 2½ per cent. solution of the sugar is condensed to methylfructoside, mainly the γ-isomeride $[\alpha]_D + 26°$. This decolorises permanganate and is very readily hydrolysed by acids. It is converted on methylation into the same tetramethyl-γ-fructose as has been obtained from sucrose and from inulin on hydrolysis.

Crystalline β-methylfructoside, $[\alpha]_D - 172°$, was obtained by Hudson and Brauns [38] by methylation of tetracetylfructose and subsequent hydrolysis. It is not hydrolysed by the enzymes of yeast nor by emulsin and does not show mutarotation. The crystalline α-methylfructoside $[\alpha]_D + 44°$ has been prepared by Schlubach.[39]

α-Tetracetylchlorofructose is more stable in solution than the corresponding acetochloroglucose: to convert it into the glycoside its ethereal solution is added to silver nitrate in methyl alcohol in presence of pyridine. It is proved to belong to the pyranose series by conversion *via* pentamethylfructoside into 1 : 3 : 4 : 5-tetramethylfructose.

The α- and β-forms have been prepared by Brauns [40] and further studied by Hudson.

Hudson deduces from their rotations that neither α-pentacetylfructose nor β-acetochlorofructose $[\alpha]_D + 45°$ have normal structure, a supposition which has been confirmed by Pacsu.[41] α-Pentacetylfructose must contain a free ketose group since on reduction and acetylation it is converted into a mixture of the two possible alcohols, viz. hexacetylsorbitol and mannitol.

Similarly, the chlorine in α-acetochlorofructose is not replaceable by silver acetate in acetic anhydride, and it behaves as if it were substituted on $C_6$.

The two pentacetates have been made, the lævorotatory β from fructose in the customary manner, and the dextrorotatory α only from the tetracetate.[42]

Tetracetylchloro-γ-fructose, which is of such importance as an intermediate for the desired synthesis of sucrose, was prepared by Irvine, Oldham and Skinner [43] by the following series of reactions:

Fructose → γ-ethylfructoside → tetracetyl-γ-ethylfructoside → tetracetylchloro-γ-fructose → tetracetyl-γ-fructose.

At every stage the structure of the compounds as furanose sugars was confirmed by conversion into tetramethylfructose.

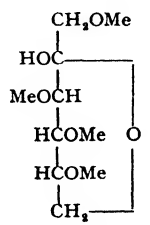
Tetracetyl-γ-fructose $[\alpha]_D + 38{\cdot}7°$ in benzene (+ 31·5° in chloroform) is also conveniently obtained from inulin.[44] It is isomeric with the crystalline tetracetyl fructoses of the stable pyranose type

$[\alpha]_D - 92°$. As it is a syrup it has been sought to prepare it by different methods and from different sources so as to obtain products which were both identical among themselves and the same as the product of the hydrolysis of sucrose octacetate.[45] There is the possibility that the compound may be a mixture of different ring types. As is described later, in no case has the combination of this fructose derivative with tetracetylglucose given rise to a derivative of sucrose. One acetyl group in this compound is more stable towards cold alkali than the other three.

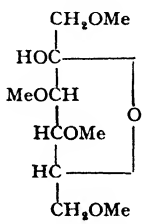
$\gamma$-Methylfructoside 2 : 5, obtained by Menzies[46] by condensing fructose at room temperature with dry methyl alcohol carefully freed from acetone, is only known as a syrup $[\alpha]_D + 26°$. It forms stable, crystalline tetracarbomethoxy derivatives.[47]

This $\gamma$-fructoside is a mixture of two stereoisomerides and contains also some quantity of normal fructosides. What is interesting is that it is hydrolysed by yeast invertase at about the same rate as sucrose, thus correlating the action of this enzyme with $\gamma$-fructofuranose in each case.[48]

*The Methyl Fructoses.*—The isomeric tetramethyl-fructoses have been specially studied[49] on account of their importance as reference compounds which serve to determine the particular type to which a fructose derivative belongs and the structure of the polysaccharides derived from fructose. Two types are known, distinguished as $\alpha$- and $\beta$-. The crystalline $\alpha$-isomeride, melting at 98°-99°, prepared from tetracetylfructose is lævorotatory and undergoes mutarotation very rapidly to $[\alpha]_D - 123°$, but it is otherwise stable: it condenses very slowly with methyl alcohol in presence of 0·25 per cent. of hydrogen chloride, taking seventy-two days for complete conversion as compared with the twenty-four hours required by the $\beta$-isomeride obtained from sucrose. The fructoside formed by the latter is also much more readily hydrolysed than $\beta$-methylfructoside. 1 : 3 : 4 : 5-Tetramethylfructose 2 : 6, the $\alpha$-isomeride, has had its constitution definitely confirmed,[50] by conversion into *d*-araboglutaric acid.




1 : 3 : 4 : 5—(2 : 6)
Tetramethylfructose



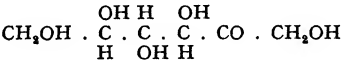

1 : 3 : 4 : 6—(2 : 5)
Tetramethylfructose

1 : 3 : 4 : 6-Tetramethylfructose, the $\beta$-isomeride, is a syrup $[\alpha]_D$ + 31°.[51] It was isolated from heptamethylsucrose [52] and proved to have the above constitution (*q.v.* sucrose).

The 3-monomethylfructose from fructose monoacetone contains the methyl group in position 3. It yields a crystalline methylfructoside $[\alpha]_D$ — 34·6°.[53] It is also produced on enolisation of 3-methylglucose.

Normal fructose has but one primary $CH_2OH$ group, but there are two in $\gamma$-fructose which have different properties, only one *p*-toluenesulphonyl residue attached in this way being capable of replacement by iodine. This is that on $C_6$, since the aromatic group on $C_1$ in both forms of fructose does not react with sodium iodide.

## Sorbose:



Sorbose, $[\alpha]_D$ — 43·2°, m.p. 159°-161°, was discovered by Pelouze in 1852 and was isolated from the juice of mountain-ash berries which had been exposed to the air for many months. These berries contain the alcohol sorbitol, which, under the influence of an oxidising organism, shown by Emmerling to be identical with the *Bacterium xylinum* of Adrian Brown, is oxidised to sorbose. Sorbose is therefore not a primary plant product, but is formed by secondary bacterial oxidation.

The brilliant researches of Bertrand [54] have given a complete explanation of the transformation, and have rendered the preparation of sorbose a relatively simple matter. According to Schlubach and Vorwerk [55] sorbose can now be prepared readily in 50-75 per cent. yield by the action of *B. xylinum* on sionon, a commercial sorbitol preparation. It is advisable to add ·5 per cent. acetic acid to the cultures to restrain the growth of other organisms. Generally it behaves as fructose, but it has a marked crystallising power and is not fermentable; on reduction it yields sorbitol and iditol. Lobry de Bruyn has shown that under the influence of alkali sorbose is partially converted into *l*-gulose, *l*-idose and *l*-galactose.

Fischer originally designated sorbose as *d*-sorbose because on reduction it yields the same sorbitol as *d*-glucose. Both Rosanoff and Hudson pointed out that it should be *l*-sorbose on account of the stereochemical relationship to *l*-glycerose and *l*-glucose.

$\alpha$-Methyl-*l*-sorboside, $[\alpha]_D$ — 88°, obtained by the action of methyl alcohol and hydrochloric acid on *l*-sorbose, is so named to fit

in with the convention that the α-isomeride of a lævo sugar is the more lævorotatory.

Sorbose forms a pentacetate, which is considered to be 1 : 3 : 4 : 5 : 6-pentacetyl keto-*l*-sorbose.

Hudson failed to detect any mutarotation of sorbose, but according to Riiber [56] a feeble mutarotation is exhibited; aqueous solutions contain therefore almost entirely the α-form.

The antipode *d*-sorbose has been prepared by Lobry de Bruyn and van Ekenstein by the partial transformation of *d*-galactose under the influence of dilute alkalis. It has $[\alpha]_D + 42{\cdot}9°$.

## REFERENCES TO CHAPTER XI.—THE ALDO AND KETO HEXOSES.

### MANNOSE.


1. Clements, Plant Physiol., 1932, **7**, 547.
2. Clark, J.B.C., 1922, **51**, 1.
3. Manske, J.B.C., 1930, **86**, 571.
4. Nishida and Hashima, J. Dept. Agric. Kyushu., 1930, **2**, 277.
5. Fränkel and Jellinek, Biochem. Z., 1927, **185**, 392. Levene and Mori, J.B.C., 1929, **84**, 49.
6. Dische, Biochem. Z., 1928, **201**, 74. Bierry, Compt. rend., 1930, **190**, 404. Rimington, Biochem. J., 1931, **25**, 1062.
7. Anderson and Renfrew, J.A.C.S., 1930, 1252.
8. Riiber and Minsaas, Ber., 1927, **60**, 2402.
9. Levene, J.B.C., 1923, **57**, 329; J.B.C., 1924, **59**, 129.
10. Fischer and Hirschberger, Ber., 1889, **22**, 321.
11. Wolfrom and Lewis, J.A.C.S., 1928, 837.
12. Isbell, Bur. Stand. J. Res., 1930, **5**, 1179; Bur. Stand. J. Res., 1931, **6**, 1115.
13. Haworth, Hirst et alii, J.C.S., 1930, 2626; 2644.
14. Haworth, Hirst et alii, J.C.S., 1930, 2653; 1931, 1349; 1354.


### GALACTOSE.


15. Pasteur, Compt. rend., 1856, **42**, 347.
16. Lippmann, Ber., 1910, **43**, 3611.
17. Anderson, J.B.C., 1933, **100**, 244.
18. Winterstein, Ber., 1888, **31**, 1571.
19. Oshima and Tollens, Ber., 1901, **34**, 1422.
20. Pryde and Humphreys, Biochem. J., 1926, **20**, 825.
21. Klenk, Z. Physiol. Chem., 1931, **198**, 25. Thierfelder and Klenk, Die Chemie der Cerebroside und Phosphatide, Berlin, 1930.
22. Riiber, Minsaas and Lyche, J.C.S., 1929, 2173. Smith and Lowry, J.C.S., 1928, 666.
23. Schlubach and Prochownick, Ber., 1929, **62**, 1502.
24. Freudenberg and Raschig, Ber., 1927, **60**, 1633.
25. Schlubach and Meisenheimer, Ber., 1934, **67**, 429.
26. Micheel and Suckfüll, Ann., 1933, **502**, 85; **507**, 138.
27. Helferich and Sparmberg, Ber., 1931, **64**, 104.
29. Micheel, Ber., 1933, **66**, 1957.

## GULOSE.

29. Fischer and Piloty, Ber., 1891, **24**, 521.
30. Fischer and Stahel, Ber., 1891, **24**, 528.
31. Isbell, Bur. Stand. J. Res., 1929, **3**, 1041; 1930, **5**, 741; 1932, **8**, 1.

## FRUCTOSE.

32. Haworth, Hirst and Learner, J.C.S., 1927, 1040. Goodyear and Haworth, J.C.S., 1927, 3136.
33. Schlubach and Rauchalles, Ber., 1925, **58**, 1842.
34. Irvine and Robertson, J.C.S., 1916, 1305.
35. Haworth and Linnell, J.C.S., 1923, 294.
36. Hudson, J.A.C.S., 1930, 1680; 1715.
37. Avery, Haworth and Hirst, J.C.S., 1927, 2038. Haworth, Hirst and Nicholson, J.C.S., 1927, 1513. Haworth, Hirst and Learner, J.C.S., 1927, 2432.
38. Hudson and Brauns, J.A.C.S., 1916, 1216.
39. Schlubach and Schröter, Ber., 1928, **60**, 1216.
40. Brauns, J.A.C.S., 1920, 1846. Hudson, J.A.C.S., 1924, 477.
41. Pacsu, J.A.C.S., 1932, 1697.
42. Hudson and Brauns, J.A.C.S., 1915, 1283; 2736.
43. Irvine, Oldham and Skinner, J.A.C.S., 1929, **51**, 1279.
44. Steele, J.C.S., 1918, 261.
45. Irvine and Stiller, J.A.C.S., 1932, 1079.
46. Menzies, J.C.S., 1922, 2238.
47. Allpress, Haworth and Inkster, J.C.S., 1927, 1233.
48. Schlubach and Rauchalles, Ber., 1925, **58**, 1842.
49. Irvine and Patterson, J.C.S., 1933, 2159.
50. Haworth, J.C.S., 1926, 1858; 1927, 1040; 1929, 1337.
51. Haworth, J.C.S., 1927, 2308; 2432.
52. Haworth, J.C.S., 1920, 199.
53. Allpress, J.C.S., 1926, 1720.

## SORBOSE.

54. Bertrand, Compt. rend., 1898, **127**, 728.
55. Schlubach and Vorwerk, Ber., 1933, **66**, 1251.
56. Riiber, Tidsskr. Kjemi. Berg., 1932, **12**, 227.

## CHAPTER XII.

### THE PENTOSES AND METHYLPENTOSES.

### The Pentoses.

$C_5H_{10}O_5$.—Two pentoses, *l*-arabinose and *d*-xylose,* are widely distributed in plants. They are not known in the free condition, but occur combined with glucose and a variety of aglycones in vicianosides and primeverosides respectively, and in a number of types of polysaccharides.

Xylose is the sole product of hydrolysis of xylan which is found in that fraction of the hemicelluloses of wood which is soluble in alkalis, known as wood gum, and also in straw, maize cobs, etc. Hudson found that xylose can be very readily prepared from cotton-seed hulls with a yield of 8-12 per cent. The hulls are extracted first with 2 per cent. ammonia and then hydrolysed by boiling with 7 per cent. sulphuric acid. The filtrate is carefully neutralised with calcium hydroxide, made just acid with phosphoric acid and concentrated. The remaining calcium sulphate is precipitated on the addition of alcohol and the solution evaporated to a syrup under reduced pressure. This is mixed with alcohol and crystallisation soon takes place.

Hudson, Monroe, and latterly Ling and Nanji [1] have worked out the preparation from maize cobs, the yield being 10-12 per cent. Peanut shells and cotton-seed bran are considered practical industrial sources of xylose.[2]

Arabinose is obtained from the polymeric arabans which occur in wheat bran, fruit skins, and especially in plant gums : a homogeneous araban which yields only arabinose on hydrolysis has not been prepared. Arabinose is a constituent of pectin. It is conveniently prepared from cherry gum or gum-arabic, or from sugar-beet pulp.[3]

The *d*-isomer of arabinose occurs in plants only in the glycoside barbaloin and has been called aloinose (Léger) [4]; it was found by Anderson [5] in the polysaccharide of tubercle bacilli. These are the

* Once known as *l*-xylose.

only instance of the occurrence in nature of *d*-arabinose, which can be obtained synthetically by degradation of *d*-glucose by the methods indicated in Chapter VII.

The pentose *d*-ribose is found in the nucleosides which are components of the nucleic acids of both plants and animals. Thymonucleic acids contain nucleosides of 2-desoxyribose.[6]

The pentose which appears in the urine in the rare disease pentosuria has been identified at various times with the different pentoses by different authors, but at no time very convincingly. It is possible there may be several kinds of pentosuria. Levene and also Grindwald state that the pentose in urine is *l*-xyloketose.

The colour reactions given by the pentoses on heating with hydrochloric acid in the presence of orcinol or phloroglucinol are very characteristic, and are frequently used for their detection. Pentoses are determined quantitatively by distilling with 12 per cent. hydrochloric acid and precipitating the insoluble phloroglucinol derivative of the furfuraldehyde which is produced. The method must be used with caution since the yield is not quantitative, and a correction factor is used and a specified experimental procedure must be adhered to. Further, if methylpentoses are present, methylfurfuraldehyde is produced which also forms an insoluble phloroglucide which, however, can with some difficulty be separated by alcohol treatment,[7] as can the phloroglucide of $\omega$-hydroxy-methylfurfuraldehyde produced from hexoses.

The phloroglucinol method is unreliable with hexose rich material and does not permit determination of the methylpentoses. An alternative method is the use of barbituric acid, which forms insoluble compounds with furfuraldehyde and methylfurfuraldehyde but not with hydroxy-methylfurfuraldehyde. Pentose and methylpentose are determined together by this method.

Arabinose and xylose show the usual aldose reactions. They are not fermented by yeasts. Organisms which ferment pentoses are widely distributed in nature; for example, the acetic and lactic fermentations of silage and sauerkraut are largely due to their activity.

Arabinose forms a characteristic, almost insoluble, diphenyl hydrazone. Xylose is best recognised by conversion into xylonic acid, and isolation of this as the cadmium bromide double salt.

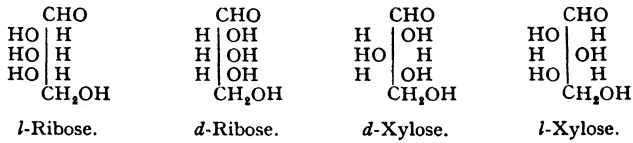
Both pentoses form the corresponding methylpentosides and these can be methylated in the usual manner.

A series of lævorotatory derivatives of *l*-arabinose belonging to

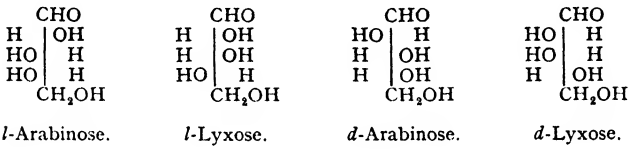
the $\gamma$-type have been isolated.[8] The corresponding $\gamma$-xylose derivatives are all dextrorotatory.[9]

The configurations of the eight possible aldopentoses are given in the following table, which also contains the formulæ of the lower members of the group of monosaccharides which remain for consideration, four tetroses and the two trioses :—

## Aldopentoses.

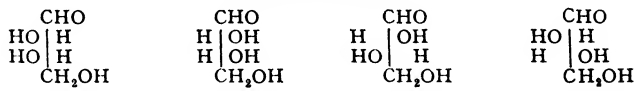



*l*-Ribose. *d*-Ribose. *d*-Xylose. *l*-Xylose.



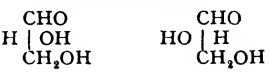

*l*-Arabinose. *l*-Lyxose. *d*-Arabinose. *d*-Lyxose.

## Aldotetroses.



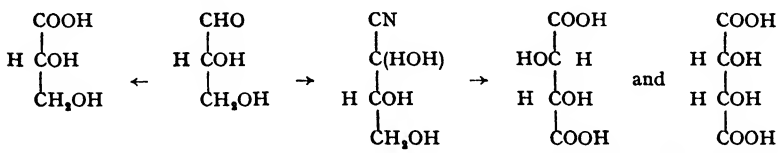

*l*-Erythrose. *d*-Erythrose. *l*-Threose. *d*-Threose.

## Aldotrioses.




*d*-Glycerose. *l*-Glycerose.

The optically active glyceroses (glyceraldehydes) were synthesised by Wohl. The dextrorotatory form has $[\alpha]_D - 13°\text{-}14°$. By the hydrogen cyanide synthesis it is converted into a trihydroxy-butyric acid which yields on oxidation a mixture of *l*-tartaric and *meso*tartaric acids : *l*-tartaric acid is the acid corresponding to *d*-threose :—




(−) Glyceric acid. *d*-(+) Glycerose. *l*-(−) Tartaric acid. *Meso*tartaric acid.

*d*-(+) glycerose gives on oxidation (—) glyceric acid. Fortunately, as pointed out on page 16, the designation *d*-glycerose expresses both the sign of the optical rotation and the spatial relationship with *d*-glucose.

When the cyanhydrin synthesis is applied to natural *l*-arabinose a mixture of two nitriles is obtained, and the corresponding acids, when reduced, give rise to *l*-glucose and *l*-mannose; similarly, *d*-xylose can be converted into *d*-gulose and *d*-idose. *d*-Glucose, when degraded by the methods of Ruff or Wohl, gives *d*-arabinose; *d*-galactose forms *d*-lyxose. The carbon atom which requires to be eliminated in order that *d*-glucose may give rise to the natural *d*-xylose, a transformation which there is strong reason to think may take place in the plant, is not the one effected by the processes described, but is situated at the opposite end of the chain. No direct chemical means of effecting this change has as yet been discovered, though the corresponding methylpentose, *d-iso*rhamnose, has been prepared from glucose.

*Ribose.*—

$$CH_2OH \cdot \underset{OH}{C}\cdot\underset{OH}{C}\cdot\underset{OH}{C}\cdot CHO \qquad (H\ H\ H)$$

*d*-Ribose was first obtained crystalline by Levene and Jacobs.[10] In plants it is found in the simple nucleoside, crotonoside discovered by Cherbuliez and Bernhard [11] in the seeds of *Croton tiglium*, combined with isoguanine, and in plant nucleic acid which is identical with yeast nucleic acid. This nucleic acid is made up of four nucleotides, each consisting of a molecule of phosphoric acid joined to a nucleoside consisting of *d*-ribose and one of the purines, guanine or adenine, or the pyrimidines, cytosine or uracil. The compound vernine which Schulze [12] found in a number of plant extracts is identical with guanosine.

In animals the nucleotides adenylic, guanylic and inosinic acids which, on hydrolysis, yield ribose, phosphoric acid and adenine, guanine or hypoxanthine respectively, are found as extranuclear cell constituents. Inosine can be prepared from Liebig's extract of beef. A nucleoside of ribose and uric acid was found by Benedict [13] in beef blood.

In animal nucleic acid, however, in the nucleotides ribose is replaced by desoxyribose and instead of uracil is found thymine.

The study of the nucleic acids has in recent years been mainly carried out by Levene.[14]

*d*-Ribose has $[\alpha]_D$ — 19·5°, its m.p. is 86°-87° and that of its phenylosazone 163°-164°. Its structure was substantiated by its

synthesis by van Ekenstein and Blanksma.[15] Methylriboside and its derivatives have been shown to have the pyranose structure by Levene and Tipson.[16]

*d*-Ribose is the pentose corresponding to *d*-allose and *d*-altrose, both of which hexoses have been synthesised from it by Levene and Jacobs.[17]

Robinson [18] in 1927 raised the question whether the sugar ribose present in nucleosides is the same as that present in the nucleotides: he suggested that in the latter the sugar might really be *d*-xylose, and that ribose resulted from a Walden inversion following removal of the phosphoric acid group at carbon 3. The evidence now speaks against this hypothesis. Levene found that the phosphoric acid in inosinic acid is attached to carbon 5 and its removal could not cause an inversion. In other nucleotides the phosphoric acid is attached at $C_2$ or $C_3$, but the same ribose is obtained from the different nucleotides regardless of whether the hydrolysis is effected by acids, alkalis or by enzymes.

## The Methylpentoses.

There are sixteen possible methylpentoses, corresponding in configuration with the sixteen aldohexoses figured on page 13. In each the terminal or side chain $CH_2OH$ is replaced by $CH_3$. In order to bring out their relation to the hexoses Votoček has introduced a systematic nomenclature for them by suffixing the term methylose to the name of the corresponding hexose. Thus rhamnose becomes *l*-mannomethylose, *l*-fucose is *l*-galactomethylose. The existing names rhamnose, epirhamnose and fucose are retained, others such as rhodeose, chinovose and isorhamnose have become superfluous and have been abandoned. Progress in this interesting group is largely the result of the work of Votoček [19] and his pupils, and latterly also of Freudenberg and Raschig.[20]

Ten methylpentoses have been obtained crystalline; four of them occur in plants either in glycosides or as polymers; six have been synthesised in the laboratory.

The four natural methylpentoses are—

| | | | |
|---|---|---|---|
| CHO | CHO | CHO | CHO |
| H \| OH | H \| OH | HO \| H | H \| OH |
| H \| OH | HO \| H | H \| OH | HO \| H |
| HO \| H | HO \| H | H \| OH | H \| OH |
| HO \| H | H \| OH | HO \| H | H \| OH |
| $CH_3$ | $CH_3$ | $CH_3$ | $CH_3$ |
| *l*-(+) Rhamnose = *l*-mannomethylose. | *d*-(+) Fucose *d*-galacto methylose. | *l*-(−) Fucose *l*-galacto methylose. | *d*-(+) Epirhamnose *d*-glucomethylose. |

(The signs in brackets indicate the sign of the rotation.)

The natural origin of the methylpentoses in plants is obscure. *l*-Rhamnose, which is perhaps the commonest, has the same configuration as *l*-mannose, and hence cannot be derived from *d*-glucose or *d*-mannose by a simple process of reduction. Freudenberg and Raschig [21] have suggested disproportionation of the natural hexitols as a possible source, and they show how it is possible to derive *d*-epirhamnose from sorbitol, *d*- and *l*-fucose from dulcitol. But *l*-rhamnose cannot be derived in this way from natural *d*-mannitol. Another possibility is that they are derived by the fission of inositols or quercitols. At present there is no satisfactory theory for their derivation from the commoner sugars and an independent synthesis must be looked to for their origin.

Their biological significance is likewise not yet understood; they are not fermented by yeasts.

They show most of the reactions characteristic of the pentoses except that they form methylfurfuraldehyde on distillation with acids.

Fischer and Zach [22] established the constitution of the methylpentoses beyond doubt by the conversion of *d*-glucose into *d*-epirhamnose. Starting from triacetyl methylglucoside bromohydrin, prepared from acetodibromoglucose by substitution of methoxyl for one bromine atom (page 113), the bromine atom was removed by means of zinc dust and acetic acid. The triacetyl derivative obtained yielded a methylglycoside on alkaline hydrolysis from which the methylpentose was finally obtained on acid hydrolysis. Since no optical inversion can have taken place during the transformation, because no asymmetric carbon atom was concerned, *d*-epirhamnose must have the same configuration as glucose, and further, *l*-rhamnose the configuration of *l*-mannose.

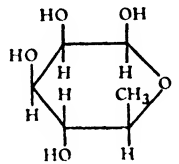
The synthetic methylpentoses are—

*d*-(—) mannomethylose or *d*-rhamnose.
*d*-(—) gulomethylose.
*d*-(+) talomethylose or *d*-epifucose.
*l*-(—) altromethylose.
*l*-(—) glucomethylose or *l*-epirhamnose.
*l*-(—) talomethylose or *l*-epifucose.

Each pair of methyloses give a common phenylosazone: these have been isolated and characterised:—

| Pair. | $[\alpha]_D$. | M.p. °C. |
|---|---|---|
| *d*-Allo and altro . | − 75° | 185° |
| *d*-Gluco and manno . | − 77° | 191° |
| *d*-Gulo and ido . | left | 140° |
| *d*-Galacto and talo . | + 70° | 178° |

*l-Rhamnose = l-mannomethylose.*




*Rhamnose*, $C_6H_{12}O_5$, is a constituent of many glycosides, the best known of which are quercitrin, and xanthorhamnin, the colouring matter of Persian berries. It occurs particularly in combination with flavonol derivatives and with saponins. According to Walton [23] a commercial product " lemon flavin " is rich in rhamnose, and gives a yield of 20-25 per cent. on direct acid hydrolysis. Rhamnose is also a constituent of the polysaccharides of some of the gums and mucilages where it is associated with galacturonic acid.

Rhamnose crystallises with a molecule of water, the hydrate having the composition $C_6H_{14}O_6$; in consequence it was regarded at one time as belonging to the hexahydric alcohols and called " isodulcitol."

Rhamnose forms a phenylosazone and other derivatives similar to those of glucose. It exists in $\alpha$- and $\beta$-forms which exhibit mutarotation. By the cyanhydrin reaction two rhamnohexonic acids are formed, one of which yields mucic acid when oxidised. The synthesis has been extended to the preparation of rhamnohexose and rhamnoheptose.

Contrary to Bertrand's rule it is found that neither *l*-rhamnitol nor $\alpha$- nor $\beta$-rhamnohexitol is oxidised by sorbose bacteria,[24] although mannitol is readily attacked, and hence it is concluded that attack by this culture does not depend only on stereochemical configuration, but also on the homologous series to which the alcohol belongs.

While rhamnose is not fermented by yeasts it is metabolised by bacteria, and a bacterium from human fæces is able to utilise rhamnose but no other sugar, and has been therefore suggested as a microbiological test for rhamnose.[25]

Rhamnose, containing two *cis* hydroxyl groups next to the

aldehyde carbon has a similar structure to mannose, and its behaviour in some respects parallels that of mannose and lyxose.

*Epirhamnose = glucomethylose.*—*l*-Epirhamnose was originally obtained by Fischer and Herborn [26] by heating rhamnonic acid with pyridine and reduction of the epimeride. *d*-Epirhamnose along with altromethylose has been prepared from the unsaturated galactoseen.

*d*-Epirhamnose is a constituent of the glycoside convolvulin. Chinovose (or quinovose), the sugar component of the glycoside chinovin obtained from *Cinchona* bark, has been shown to be identical with *d*-epirhamnose.[27] The name can, therefore, be deleted from the literature.

*d- and l-Fucose = galactomethylose.*—*l*-Fucose occurs as its polymeride fucosan, which is a component of the cell walls of marine algæ. Clarke [28] has described its preparation from *Ascophyllum nodosum* by hydrolysis with 2 per cent. sulphuric acid. It is isolated by means of the phenylhydrazone, which is then decomposed with benzaldehyde. Forty grams of *l*-fucose were obtained from 10 kg. of air-dried seaweed. The crystalline sugar has $[\alpha]_D - 75{\cdot}5°$. Clarke established its configuration by converting it into a strongly lævorotatory methyltetronolactone, $[\alpha]_D - 63{\cdot}65°$, and its amide $[\alpha]_D + 18{\cdot}48°$.

*l*-Epifucose (= *l*-talomethylose) obtained from epifuconic acid, and its derivatives are described by Votoček and Kučerenko.[29]

*d*-Fucose, m.p. 140°, $[\alpha]_D + 75{\cdot}7°$ (final value), sometimes called rhodeose, has been found only in the glycosides convolvulin and turpethin.

*d*-Fucose has been made from galactose via the toluenesulphonyl derivative of diacetone galactose. Sodium iodide converts the $CH_2OH$ group to $-CH_2I$ which is reduced by sodium.[30] Votoček and Valentin [31] have also prepared its epimer *d*-talomethylose.

## Other Monosaccharides.

*Apiose.*—Mention may be made of an altogether abnormal sugar, termed apiose,[32] on account of its presence in the flavone glycoside apiin found in parsley. This contains a branched chain of carbon atoms, having the formula

$$\begin{matrix} CH_2OH \\ CH_2OH \end{matrix}\!\!\rangle C(OH) \cdot CH(OH) \cdot CHO$$

It is not fermentable. Bromine oxidises it to apionic acid; when reduced by hydrogen iodide and phosphorus, isovaleric acid is

obtained. Apiin contains the disaccharide glucoapiose; when hydrolysed by dilute mineral acids apiose and glucoapigenin are formed, and the latter can be split by emulsin to glucose and apigenin. Apiin itself is not hydrolysed by emulsin.

*Hamamelose.*—

$$CH_2OH \cdot CHOH \cdot CHOH \cdot C(OH)\begin{cases} CH_2OH \\ CHO \end{cases}$$

This sugar was discovered by Freudenberg in the crystalline Hamameli tannin from the bark of *Hamamelis virginica.* Each molecule of sugar bears two of gallic acid, which can be removed by the action of tannase.

Hamamelose is an aldohexose yielding a phenylhydrazone but no osazone, proving the aldehyde group to be attached to a tertiary carbon. It is oxidised to an acid containing the same number of carbons which on reduction yield methyl propyl acetic acid. The sugar forms a methylglycoside which hydrolyses readily, for which a furanose structure is proposed by Schmidt.[33]

The crystalline tannin has nine hydroxyl groups. After conversion into its methylglycoside it is hydrolysed to gallic acid and methylhamameloside.[34]

*Digitoxose.*—This remarkable sugar, obtained from the heart-specific glycosides of the leaves of the foxglove, was first shown by Kiliani [35] to be a reduced methylpentose and finally by Micheel [36] to have the following configuration :—

$$CH_3 \cdot \underset{OH}{\overset{H}{C}} \cdot \underset{OH}{\overset{H}{C}} \cdot \underset{OH}{\overset{H}{C}} \cdot CH_2 \cdot CHO$$

It is thus the desoxymethylpentose related to the aldohexoses *d*-allose and *d*-altrose, and is unrelated stereochemically to any of the natural hexoses.

It crystallises in prisms, m.p. 106°, $[\alpha]_D + 46°$.

It has become of increased interest since the discovery of desoxyribose as a constituent of thymus nucleic acid.

*Cymarose.*—This sugar is present in the strophanthidin glycoside, cymarin, present in Canadian hemp, and also in periplocymarin from *Periploca græca.* It is represented provisionally as a methyl ether of digitoxose, [37]

$$CH_3 \cdot CHOH \cdot CHOH \cdot CHOMe \cdot CH_2 \cdot CHO$$

*Sarmentose.*—Jacobs and Bigelow [38] found the methyl ether of a desoxy sugar could be obtained in crystalline form from sarmento-

cymarin of *Strophanthus sarmentosus*. Isomeric with cymarose $C_7H_{14}O_4$, it melts 78°-79°, has $[\alpha]_D^{20°} + 12°$, increasing in twenty-four hours to 15·8°.

*Digitalose.*—The sugar derived from digitalin and perhaps oleandrin is considered by Kiliani [39] to have the structure of a methoxy methylpentose and not that of a desoxy sugar. Provisionally it is given the following formula :—

$$CH_3 \cdot CH(OH) \cdot \overset{\overset{OH}{|}}{C}\overset{\overset{H}{|}}{} \cdot CH(OH) \cdot CH(OMe) \cdot CHO$$

It reacts as an aldose but does not form an osazone.

## REFERENCES TO CHAPTER XII.

### THE PENTOSES.


1. Hudson and Harding, J.A.C.S., 1918, 1601. Monroe, J.A.C.S., 1919, 1002. Ling and Nanji, J.C.S., 1923, 620. Harding, Sugar, 1923, **25**, 124.
2. Emley, Ind. Eng. Chem. News, Edition 1928, **6**, No. 21, p. 3.
3. Harding, Sugar, 1922, **24**, 656.
4. Léger, Compt. rend., 1910, **150**, 1695.
5. Anderson, Z. Physiol. Chem., 1930, **191**, 172.
6. Levene and Mori, J.B.C., 1929, **83**, 803.
7. Klingstedt, Z. Anal. Chem., 1925, **66**, 124.
8. Baker and Haworth, J.C.S., 1925, 365.
9. Haworth and Westgarth, J.C.S., 1926, 880.
10. Levene and Jacobs, Ber., 1909, **42**, 1198.
11. Cherbuliez and Bernhard, Helv. Chim. Acta, 1932, **15**, 464.
12. Schulze and Bosshard, Z. Physiol. Chem., 1885, **9**, 443 ; Z. Physiol. Chem., 1886, **10**, 80.
13. Davis, Newton and Benedict, J.B.C., 1922, **54**, 595.
14. Levene and Bass, *The Nucleic Acids*, New York, 1931.
15. van Ekenstein and Blanksma, Chem. Weekblad., 1913, **10**, 664.
16. Levene and Tipson, J.B.C., 1931, **93**, 623.
17. Levene and Jacobs, Ber., 1910, **43**, 3141.
18. Robinson, Nature, 1927, **120**, 44 ; 656.


### THE METHYLPENTOSES.


19. Votoček, Bull. Soc. Chim., 1928, **43**, 1.
20. Freudenberg and Raschig, Ber., 1928, **61**, 1750.
21. Freundenberg and Raschig, Ber., 1929, **62**, 373.
22. Fischer and Zach, Ber., 1912, **45**, 3761.
23. Walton, J.A.C.S., 1921, 127.
24. Votoček, Coll. Czech. Chem. Comm., 1930, **2**, 402.
25. Castellani, Ann. Inst. Pasteur, 1931, **47**, 297.
26. Fischer and Herborn, Ber., 1896, **29**, 1961.
27. Votoček and Rac, Coll. Czech. Chem. Comm., 1929, **4**, 239.
28. Clarke, J.B.C., 1922, **54**, 65.
29. Votoček and Kučerenko, Coll. Czech. Chem. Comm., 1930, **2**, 47.

30. FREUDENBERG AND RASCHIG, Ber., 1927, **60**, 1633.
31. VOTOČEK AND VALENTIN, Coll. Czech. Chem. Comm., 1930, **2**, 36.

## RARE SUGARS.

32. VONGERICHTEN, Ann., 1901, **318**, 121 ; Ann., 1902, **321**, 71 ; Ber., 1906, **39**, 235. SCHMIDT, Ann., 1930, **483**, 115.
33. SCHMIDT, Ann., 1929, **476**, 250.
34. FREUDENBERG AND BLÜMMEL, Ann., 1924, **440**, 45.
35. KILIANI, Ber., 1905, **38**, 4040 ; Ber., 1916, **49**, 709.
36. MICHEEL, Ber., 1930, **63**, 347.
37. WINDAUS AND HERMANNS, Ber., 1915, **48**, 479. JACOBS AND HOFMANN, J.B.C., 1926, **67**, 609 ; J.B.C., 1926, **69**, 153 ; J.B.C., 1928, **79**, 531.
38. JACOBS AND BIGELOW, J.B.C., 1932, **96**, 355.
39. KILIANI, Ber., 1916, **49**, 709.

## CHAPTER XIII.

### Carbohydrate Alcohols.

SEVERAL carbohydrate alcohols are widely distributed in plants. They crystallise well and are soluble in water. On cautious oxidation they give in turn a reducing sugar, monobasic acid and dibasic acid. They are not fermentable by yeast, though attacked by a variety of bacteria and moulds.

*Erythritol*—

$$CH_2(OH).\overset{OH}{\underset{H}{C}}.\overset{OH}{\underset{H}{C}}.CH_2(OH)$$

is found in many lichens, particularly in *Roccella* species, where it is present either free or as the ester erythrin, $C_{20}H_{22}O_{10}$, a diorsellinate of erythritol: it also occurs free in algæ and fungi.

It has a sweet taste and is optically inactive, being the meso variety. It has been synthesised from butadiene-$\gamma$-$\delta$-oxide by Pariselle. The insoluble dibenzylidene derivative is characteristic. Yeasts are without action on it: B. xylinum transforms it into a keto-tetrose.[1]

The two optically active varieties $[\alpha]_D \pm 4{\cdot}4°$ have been obtained by alkaline reduction of *l*-threose and *d*-erythrulose.

Theoretically four pentose alcohols are possible: two meso forms, viz. adonitol and xylitol; and *d*- and *l*-arabitol, obtainable by reduction of *d*- and *l*-arabinose or *l*- and *d*-lyxose.

*d-Arabitol* has been found in the fungus, *Ustilago virens*, which infects rice in Japan,[2] and in the lichen, *Lobario pulmonario*.[3]

*Adonitol*—

$$CH_2(OH).\overset{OH}{\underset{H}{C}}.\overset{OH}{\underset{H}{C}}.\overset{OH}{\underset{H}{C}}.CH_2(OH)$$

corresponds to the riboses from which it is obtained on reduction; it is found in *Adonis vernalis*.[4]

There are ten possible stereoisomeric modifications of the hexose alcohols, but three only are of interest as natural products. The others are obtainable by reduction of the appropriate aldo- or keto-hexoses.

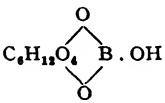
*d-Mannitol*—

$$CH_2(OH).\overset{OH}{C}.\overset{OH}{C}.\overset{H}{C}.\overset{H}{C}.CH_2(OH)$$

has been found in manna, in the sap of the larch, etc., in leaves, in fruits and roots of many families, including algæ, lichens, and bacteria, and particularly in the fungi where it exceeds glucose in quantity or even replaces it entirely. It is a normal constituent of silage, being formed there by the bacterial reduction of fructose formed from sucrose.

Mannitol seems in many cases to be a secondary putrefaction product derived from trehalose so that its formation may be avoided by preserving plant extracts under sterilised conditions. Irvine has noted specimens of sea-weed (*Laminaria*) which had become encrusted with mannitol on the surface of the thallus after the cessation of active bacterial action.

Mannitol is optically inactive in water, but becomes dextrorotatory on the addition of borax, if the mixture be acid. In alkaline solution it becomes lævorotatory. Mannitol and boric acid combine with the elimination of two molecules of water to form a ring compound mannitoboric acid, of the form



More complicated unstable compounds probably also exist in solution.

Gilmour has shown that whilst sodium hydroxide has little effect on the rotation of sodium mannitoborate and boric acid reduces it, the combination of both as sodium metaborate causes an increase which is most pronounced on the addition of one molecular proportion. The rotation of mannitol as sodium mannitoborate is $[\alpha]_D + 22{\cdot}1°$, in sodium metaborate solution the maximum obtained was $[\alpha]_D + 37{\cdot}6°$.

The rotations of all the sugar alcohols which are all of small magnitude in water, are considerably increased in borax solutions, and not infrequently reversed in sign.

*Polygalitol* [5] has, according to Shinoda and Sato, the structure of 1 : 5-anhydromannitol. It was first found by Chodat [6] in *Polygala amara*, and is now known in *P. vulgaris* and *P. tenuifolia*.[7] It melts 142°-143°, tastes sweet at first, then faintly bitter, has $[\alpha]_D + 47{\cdot}8°$ and forms a tetracetate. It is isomeric with styracitol.

*d-Sorbitol*—

$$CH_2(OH).\overset{OH}{C}.\overset{OH}{C}.\overset{H}{C}.\overset{OH}{C}.CH_2(OH)$$

corresponds to *d*-glucose, from which it is formed by reduction : it is found in the fruits and also in the leaves of most of the *Rosaceæ*, and it is easily prepared from mountain-ash berries in which it increases in amount up to one-third of the total solid material during the ripening period. It has been found as a crystalline efflorescence on the heads of the fungus *Boletus borinus*.

*l-Iditol* [8] is found in the mother liquors from mountain-ash berries but is probably of secondary origin.

When *d*-sorbitol is oxidised by means of bromine in sodium carbonate solution, the four products required by stereochemical theory, viz. *d*-glucose, *d*-fructose, *l*-gulose and *l*-sorbose, are all obtained and may be separated by the difference in the solubilities of their osazones in acetone.[9] The gulosazone (= sorbosazone) is exceedingly soluble, and can be extracted from the evaporated mother-liquor with 25 per cent. alcohol.

*d*-Sorbitol is marketed as a substitute for sugar under the name of Sionon ; it appears that in limited quantity it can be tolerated in illness.[10]

*Styracitol* [11]—$C_6H_{12}O_5$, m.p. 157°, $[\alpha]_D$—49·4° (water)—isolated from the peel of *Styrax obassia* by Asahina, was proved by Zervas [12] to be 1 : 5-anhydrosorbitol. He was able to synthesise it by reduction of tetracetyl hydroxyglucal : the appearance of the 1 : 5-oxide ring in the alcohols is of interest, possibly indicating an intermediate stage in the synthesis of sorbitol from glucose.

*Dulcitol*—

$$\begin{array}{l} \quad\ \ H\ OH\ OH\ \ H \\ CH_2(OH).C\ .\ C\ .\ C\ .\ C.CH_2(OH) \\ \quad\ \ OH\ H\ \ H\ OH \end{array}$$

is the alcohol from galactose and is found particularly in *Scrophulariaceæ* and *Celastraceæ* and also in red algæ. It is optically inactive.

| Alcohol. | Melting-point. | Optical Rotatory Power. | |
|---|---|---|---|
| | | $[\alpha]_D$ Water. | $[\alpha]_D$ Borax Solutions. |
| Erythritol . . . . | 120° | inactive | |
| Adonitol . . . . | 102° | inactive | |
| Mannitol . . . . | 166° | − 0·49° | + 20·0° |
| Dulcitol . . . . | 188° | inactive | |
| Sorbitol . . . . | 110° | − 1·73° | + 1·4° |
| Iditol . . . . | 73° | − 3·5° | |
| Persitol . . . . | 188° | slight − | + 4·5° |
| Volemitol . . . . | 153° | + 2·2° | + 22·0° |

The natural heptitols, volemitol and persitol (perseitol). are discussed on page 146 on account of their relation to the heptoses.

The physical properties of the naturally occurring sugar alcohols are summarised in the table (p. 144).

These alcohols are conveniently isolated, purified and characterised by means of their benzylidene derivatives prepared by condensing with benzaldehyde in presence of 50 per cent. sulphuric acid. The number of molecules of aldehyde concerned in the condensation varies with the configuration of the alcohol.[13]

| Alcohol. | Benzylidene Compound. | Melting-point. | $[\alpha]_D$. |
|---|---|---|---|
| *i*-Erythritol . . . | Di | 201° | — |
| Adonitol . . . . | Di | 164° | — |
| Mannitol . . . . | Tri | 213°-218° | − 13° ($CHCl_3$) |
| Dulcitol . . . . | Di | 215°-220° | |
| Sorbitol . . . . | Mono | 175° | + 29° (Acetone) |
| | Di | 163° | |
| Iditol . . . . | Tri | 219°-223° | + 6° (Acetone) |
| Persitol . . . . | Di | 219° | − 60° (Acetone) |
| Volemitol. . . . | Tri | 214° | − 1·7° ($CHCl_3$) |

These alcohols also give condensation products with acetone.

The pairs of isomeric heptitols from mannose, glucose, galactose and gulose have all been synthesised, and the synthesis carried further to the preparation of manno- and galacto-octitols, and a glucodecitol: these higher alcohols have little biological significance.

## Heptoses and Heptitols.

There are no naturally occurring aldoheptoses known, though a number have been prepared by the cyanhydrin synthesis by Fischer and others.

Two of the sixteen possible 2-ketoheptoses or heptuloses occur in nature, and two of the sixteen possible alcohols known as heptitols formed by the reduction of the heptoses are also found: in addition there are known a number of synthetic heptoses and heptitols. It is convenient to consider the heptoses and heptitols together, in order the better to appreciate their stereochemical inter-relationships.

*d-Mannoketoheptose* was isolated by La Forge [14] from the avocado or alligator pear (*Persea gratissima*). It does not exhibit mutarotation and is not fermented by yeast. The phenylosazone is identical with that of *d*-mannoaldoheptose formed by the cyanhydrin reaction from mannose: this identity establishes the structure of mannoketoheptose.

On reduction with sodium amalgam α- and β-*d*-mannoheptitol are formed, of which the former is identical with the naturally occurring persitol and the latter is identical with volemitol.

*Persitol*, or α-mannoheptitol, is obtained from the seeds of *Persea gratissima* or *P. dymifolia*.[15]

*Perseulose*.—Persitol is oxidised by *B. xylinum*, the sorbose bacterium, to a crystalline, sweet-tasting ketose, which shows mutarotation.[16] It has the configuration of *l*-galactoheptulose. On reduction perseulose gives persitol and a new alcohol, perseulitol, which is β-*l*-galaheptitol, synonymous with α-*l*-guloheptitol.

*Volemitol*, or β-*d*-mannoheptitol, is present in the fungus *Lactarius volemus*.[17] It is also identical with primulitol, which is found in the roots of Primula species, notably *P. officinalis*,[18] *elatior*, *grandiflora*, and α-sedoheptitol formed by reduction of the naturally occurring sedoheptose.

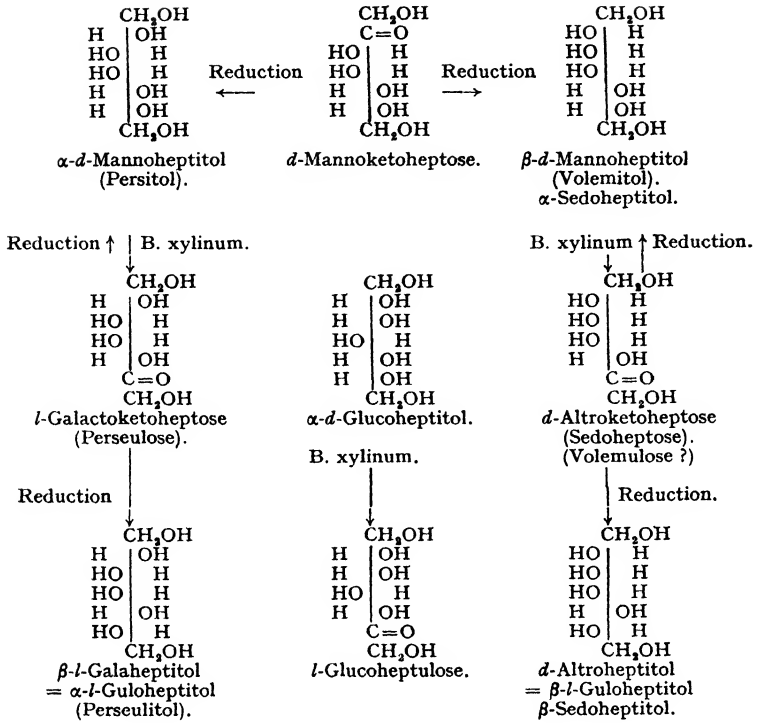
*Sedoheptose* was obtained by La Forge and Hudson [19] from a common stonecrop, *Sedum spectabile*, as a syrup. It is not fermentable. It reduces Fehling's solution, and forms a phenylosazone and is therefore a 2-ketoheptose. On reduction it yields α- and β-sedoheptitol, of which the former is identical with volemitol or β-*d*-mannoheptitol.[20]

According to Ettel [21] sedoheptose possesses the structure of *d*-altroheptulose, and therefore β-sedoheptitol is *d*-altroheptitol synonymous with β-*l*-guloheptitol.

β-sedoheptitol is remarkable in that in spite of having an asymmetric molecule, optical activity cannot be detected either in aqueous solution or in borax. The same holds for its antipode β-*d*-guloheptitol.

Volemitol is oxidised by *B. xylinum*, according to Bertrand,[22] to a ketose volemulose, which is a syrup and should be identical with sedoheptose, though this does not appear to have been specifically proved.

The configurations of these heptoses and heptitols is given in the following scheme. It is important to note how the four naturally occurring substances, mannoheptulose, sedoheptose, persitol and volemitol, are simply derived from *d*-mannose, although their systematic names relate them to sugars which do not occur in nature. Their natural origin may well be due to some such simple system of reduction and oxidation.




A characteristic of sedoheptose is that on boiling a dilute acid solution, it is partially converted into an anhydro sedoheptose. The equilibrium mixture contains 80 per cent. of this sedosan which can be obtained crystalline, $C_7H_{12}O_6$, is sweet tasting and has $[\alpha]_D - 146°$: it does not mutarotate, and has no reducing properties. Hibbert and Anderson [23] have assigned the structure of a 2 : 7-anhydropyranose to sedosan which contains one free primary $CH_2OH$ group, as evidenced by the formation of a triphenylmethyl ether.

*Glucoheptulose.—B. xylinum* oxides the optically inactive α-glucoheptitol obtained from glucoheptose prepared by the cyanhydrin reaction to a ketose for which the two configurations *d*- or *l*-glucoheptulose are possible,[24] according to which end of the molecule is selected for attack. Only one heptulose has been isolated which has m.p. 173°, $[\alpha]_D - 67{\cdot}1°$ and is called *l*-glucoheptulose, as it appears to be the optical antipode of *d*-glucoheptulose, m.p. 171°, $[\alpha]_D + 67{\cdot}6°$, which was prepared by Austin [25] from synthetic α-*d*-glucoheptose by the Lobry de Bruyn conversion with the use of lime.

*d*-glucoheptulose is unfermentable by yeast. It does not mutarotate, and forms the same osazone as *d*-glucoheptose.

On reduction *l*-glucoheptulose yields a mixture of α-glucoheptitol and a new heptitol called α-glucoheptulitol, m.p. 144°, $[\alpha]_D^{20°}$ — 2·24°, which is not identical with β-glucoheptitol.

α-glucoheptulitol should have the structure

$$\begin{array}{l} \quad\ \ OH\ \ H\quad\ OH\ \ H\quad\ H \\ HOH_2C\ .C\ .\ C\ .\ C\ .\ C\ .\ C\ .CH_2OH \\ \quad\ \ H\ \ \ OH\ \ H\quad\ OH\ \ OH \end{array}$$

and on oxidation with *B. xylinum* should yield a new ketose *l*-idoheptulose, but in fact *l*-guloheptulose is obtained once more.[26]

An inconsistency is revealed, which may mean that the interpretation of structure is incorrect or alternatively that an exception to Bertrand's rule for the method of attack of *B. xylinum* has been discovered.

The heptoses and heptitols derived from mannose and galactose have been studied particularly by Pierce.[27] α-*d*-Mannoheptitol and α-*d*-galactoheptitol are optical antipodes, the former, which is persitol, being the mirror image of the latter.

## REFERENCES TO CHAPTER XIII.


1. Bertrand, Compt. rend., 1898, **126**, 762.
2. Yabuta and Sumiki, J. Agric. Chem. Soc. Japan, 1933, **9**, 492.
3. Nolan and Keane, Nature, 1933, **132**, 281.
4. Podwyssotzki, Arch. Pharm., 1889, 141.
5. Shinoda and Sato, Ber., 1932, **65**, 1219.
6. Chodat, Arch. Sci. phys. nat., 1888, **20**, 593.
7. Picard, Bull. Soc. Chim. biol., 1929, **9**, 692.
8. Bertrand, Bull. Soc. Chim., 1906, **33**, 166.
9. Votoček and Lukes, Rec. trav. chim., 1925, **44**, 345.
10. Chem. and Ind., 1929, **48**, 573.
11. Asahina, Ber., 1912, **45**, 2363.
12. Zervas, Ber., 1930, **63**, 1689.
13. Fischer, Ber., 1894, **27**, 1524.
14. La Forge, J.B.C., 1916, **28**, 511.
15. Maquenne, Ann. Chim. Phys., 1890, **19**, 5; Weatherby and Sorber, Ind. Eng. Chem., 1931, **23**, 1421.
16. Bertrand, Compt. rend., 1908, **147**, 201.
17. Ettel, Coll. Czech. Chem. Comm., 1929, **1**, 288.
18. Bougoult and Allard, Compt. rend., 1902, **135**, 796.
19. La Forge and Hudson, J.B.C., 1917, **30**, 61.
20. Ettel, Coll. Czech. Chem. Comm., 1932, **4**, 504.
21. Ettel, Coll. Czech. Chem. Comm., 1932, **4**, 513.
22. Bertrand, Compt. rend., 1908, **147**, 201; Compt. rend., 1909, **149**, 225.
23. Hibbert and Anderson, Canadian J. Res., 1930, **3**, 306.
24. Bertrand and Nitzberg, Compt. rend., 1928, **186**, 1172.
25. Austin, J.A.C.S., 1930, 2106.
26. Khouvine (Mme.) and Nitzberg, Compt. rend., 1933, **196**, 218.
27. Pierce, J.B.C., 1916, **23**, 327.

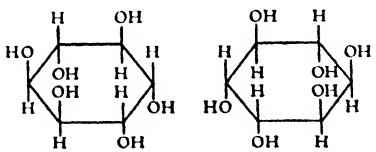
# CHAPTER XIV.

## INOSITOL AND THE CYCLITOLS.*

THE molecular formula of the aldoses is possessed also by certain cyclic polyalcohols which are hexahydro polyhydroxy benzenes. Thus the aldohexoses and ketohexoses $C_6H_{12}O_6$ are isomeric with the inositols, and the methylpentoses $C_6H_{12}O_5$ are isomeric with the quercitols.

These substances, a number of which are found in nature associated with true sugars, possess a sweet taste, are water-soluble high-melting crystalline compounds, and exist in various stereoisomeric forms, some of which are optically active; so that the superficial resemblance to the sugars justifies their inclusion in a work dealing with natural carbohydrates, particularly in view of the suggestive stereochemical relations to the hexoses which have been discovered.

A consideration of the total number of possible stereoisomers of hexahydro-hexahydroxy-benzene shows them to be nine: of these, seven are optically inactive forms possessing a plane of symmetry, two are optically active and are mirror images of each other and correspond to the *d*- and *l*-forms of inositol which occur in nature.



*d*- and *l*-inositol

The absolute configuration of the enantiomorphs is not established; thus it is not known which of the two formulæ correspond to the repective forms.

The optical activity of inositol is due to molecular asymmetry rather than to an asymmetric carbon atom, and its understanding has contributed to the development of stereochemical theory.

* The term cyclose is often used, but this is to be deprecated, since the termination " ose " suggests that a carbonyl group is present, which is untrue.

There are ten possible distinct configurations of quercitol, $C_6H_7(OH)_5$, four inactive and six optically active forms, for each of which exists an enantiomorph. Only two quercitols are known, one dextrorotatory and one lævorotatory variety, which is not the antipode of the former.

### *Inositols*, $C_6H_6(OH)_6$.

Six of the nine inositols predicted by theory have been described, namely, the natural optically active forms of *d*- and *l*-inositol, two naturally occurring meso forms, *i*-inositol and scyllitol, and two other meso forms obtained by Hugo Müller by chemical treatment of *i*-inositol and named *iso*-inositol and *ψ*-inositol.

*d-Inositol* is prepared by boiling its naturally-occurring monomethyl ether, *pinitol*, $C_7H_{14}O_6$, with concentrated hydriodic acid; it crystallises in prisms which melt at 247°-248°, and have $[\alpha]_D+68°$; it does not show mutarotation.

Pinitol, m.p. 186°, $[\alpha]_D + 65{\cdot}5°$, also known as matezite or sennite, was discovered in 1856 by Berthelot in the resin of the sugar pine, *Pinus lambertiana* (Dougl.), of California and Oregon. It also occurs in the residues from the manufacture of coniferin, in senna leaves and in Madagascar rubber. Its structure was established by Maquenne.

*l-Inositol* was obtained by Tanret by demethylation of quebrachitol in 1889. It crystallises in needles which melt at 247°, $[\alpha]_D - 65°$. The monomethyl ether *quebrachitol*, m.p. 191°, $[\alpha]_D-80°$, is found in quebraché bark, and the latex of the rubber tree. Rhodes and Wiltshire [1] have explored the possibility of the commercial production of quebrachitol from the serum which is left after the acid coagulation of the rubber in the latex. A yield of 20 lbs. a day from an estate producing 90,000 tons of rubber a month can be obtained.

The racemic inositol, composed of equimolecular proportions of the *d*- and *l*-isomerides, may be prepared by crystallisation of a mixture of the latter in equal quantities, and melts at 253°. It was found, with *i*-inositol, in the fresh ripe berries of mistletoe by Tanret.

*Meso-* or *i-inositol* (dambose, nucite) is widely distributed in plants and animals; it is found in the muscles and various organs of oxen and horses and in the urine in Bright's disease. In the vegetable world it occurs in the Leguminosæ, in the leaves of asparagus, the oak, ash and walnut, and in all parts of the grape-vine, and also in many fungi. Its chief sources for extraction are walnut leaves and mistletoe. It crystallises in bunches of needles and melts at 225°. It does not reduce Fehling's solution and is not fermented by yeast,

but is attacked by certain fungi. The hexacetate forms monoclinic plates and melts at 212°.

When inositol is evaporated almost to dryness with nitric acid and then again carefully evaporated with calcium chloride solution, a rose-red solution is obtained; if ammoniacal strontium acetate is substituted for the calcium salt, a violet tint is produced (Scherer's reaction). Both these colour tests are excessively delicate, especially the latter.

H. Müller [2] found that treatment of *i*-inositol with a solution of hydrochloric or hydriodic acid in acetic acid transformed it partially into two other meso forms :—

*iso*-Inositol, crystals melting at 246°-250°, readily soluble in water, insoluble in alcohol, but soluble in boiling 50 per cent. alcohol, and tasting faintly sweet; and

$\psi$-Inositol, an amorphous or microcrystalline compound, very soluble in water, but very sparingly so in alcohol.

*Bornesitol*, m.p. 199°, $[\alpha]_D + 31{\cdot}6°$, the monomethyl ether of *i*-inositol present in Borneo rubber is converted by hydrogen iodide into methyl iodide and *i*-inositol.

*Sequoyitol*,[3] m.p. 234°, an isomeric monomethyl ether, is extracted from the dry heartwood of redwood, *Sequoyia sempervirens*, together with pinitol. Hydriodic acid yields *i*-inositol. It is optically inactive and tastes sweet.

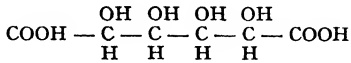
*Dambonitol*, a dimethyl ether, m.p. 195°, occurs in Gabon rubber and in the latex of *Castilloa elastica*.

*Phytin*, the calcium magnesium salt of inositol hexaphosphate, $C_6H_6[OPO(OH)_2]_6$, is an important derivative of *i*-inositol found in the seeds of many plants. It was isolated from rice bran as inositol phosphoric acid by Winterstein [4] and from maize meal by Vorbrodt. It is stable at 115°, but in presence of water at 155° is resolved into phosphoric acid and inositol; it is hydrolysed by the enzyme phytase which is widely distributed, particularly in germinating seeds.

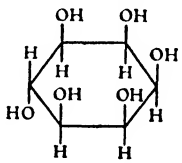
Contardi [5] synthesised an inositol hexaphosphate by the action of phosphoric acid on inositol at 120°-130° in the absence of air, and considered it to be probably identical with the phytin in seeds. Anderson [6] recorded unsuccessful attempts to obtain the hexaphosphate synthetically, obtaining only tetraphosphates and inositol derivatives of pyrophosphoric acid. S. Posternak [7] has synthesised *i*-inositol hexaphosphate.

No physiological function for phytin, other than as a phosphorus reserve, has been established.

S. and T. Posternak [8] have in all probability established the configuration of *i*-inositol. Oxidation with alkaline permanganate at 2° yields allomucic acid in small yield,



proving that at least four adjacent OH groups have the *cis* configuration. It was possible to isolate from wheat germs a lævorotatory tetraphosphate, and to make the same tetraphosphate by controlled hydrolysis of inactive hexaphosphate by phosphatase, prolonged action of which yielded active tri- and di-phosphates and the inactive monophosphate. The production of an active derivative by asymmetric removal of groups from an inactive one is somewhat novel. Consideration of the optical activity or the contrary of the phosphates enables all formulæ but one to be excluded for *i*-inositol.



This structure is interesting in view of its synthesis by Wieland and Wishart,[9] by catalytic reduction of hexahydroxy benzene; synthesis *in vitro* would normally be expected to yield a symmetrical product.

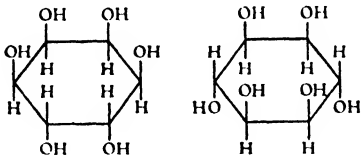
*Scyllitol*, the other natural inactive form of inositol, was formerly known by the three names of scyllitol, cocositol and quercine, but H. Müller [2] showed in 1912 that all three were identical.

Scyllitol was discovered by Staedeler and Friedrichs in 1858 in various organs of the spur dog-fish (Plagiostomi). H. Müller [10] found it in 1907 in the leaves of *Cocos nucifera* (Linn.) and *Cocos plumosa* (Hook), assigning to it at that time the name "cocositol." It also occurs in acorns. Goodson has found it in the leaves of *Helinus oratus*, a climbing shrub indigenous to S. Africa, and Sando [11] in the dogwood *Cornus florida*.

The formation of the same stereoisomer in such different organisms as those of the cocoa-nut palm and oak on the one hand, and the spur dog-fish on the other is very remarkable.

Scyllitol is optically inactive, forms hard lustrous monoclinic prisms which melt at 349°-350°, is sparingly soluble in water, gives Scherer's colour reaction and yields the customary acetyl, benzoyl, etc., esters.

On oxidation with alcoholic permanganate a small yield of mucic acid is obtained,[12] so that there are only two alternative configurations possible for inactive scyllitol.



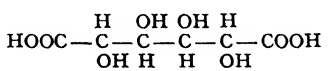
*Mytilitol*, $C_7H_{14}O_6$, m.p. 273°, which occurs in the valve muscles of *Mytilus edulis*, forms a pentacetate with acetic anhydride but a hexacetate, m.p. 182°, with acetic anhydride and concentrated sulphuric acid. It is optically inactive.

It was regarded by Ackermann as an isomeride of inositol and by Jansen as a quercitol, but Daniel and Doran [13] prefer to regard it as a methylcyclohexanhexitol.

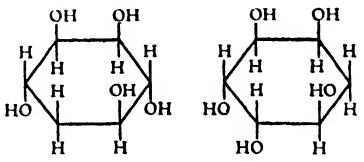
*Quercitols*, $C_6H_7(OH)_5$.—As previously mentioned, two optically active forms of the quercitols are found in plants.

*d-Quercitol* occurs in acorns and in small quantities in the cork and bark of oak. H. Müller also obtained it from the leaves of *Chamærops humilis* (Linn.), the only European representative of the palm family, which was formerly used like esparto for paper-making. The leaves contain 1·35 per cent. of quercitol.

*d*-Quercitol crystallises in prisms and melts at 234°; its rotatory power is $[\alpha]_D + 20°$. It is not fermentable. It gives pentacetates and similar esters, thus possessing five hydroxyl groups in the molecule. Oxidation with permanganate leads to the formation of malonic acid and other products which confirm its structural formula as pentahydroxycyclohexane. Oxidation with nitric acid yields mucic acid,



which Karrer [14] pointed out can only be reconciled with one of the two structures or their mirror images :—



T. Posternak [15] was able to obtain by oxidation of *d*-quercitol with cold alkaline permanganate, 19 per cent. of metasaccharonic

acid to which the configuration

$$\begin{array}{cccccccc} & & H & & OH & H & H & \\ HOOC & . & C & . & C & . C & . C & . \ COOH \\ & & OH & & H & H & OH & \end{array}$$

is ascribed. This can only arise from the first of Karrer's alternatives, and the formula of *d*-quercitol is established.

*l-Quercitol* was obtained by Power and Tutin in 1904 from the leaves of *Gymnema sylvestre* (R. Br.). It crystallises in prisms from water and in needles from alcohol and melts at 174°, having $[\alpha]_D - 74°$. It gives pentacetyl and pentabenzoyl compounds, and yields with sodium hypobromite a diketotrihydroxycyclohexane, $C_6H_5O_2(OH)_2$.

*d*- and *l*-Quercitol are not optical antipodes.

*Betitol*, $C_6H_8(OH)_4$, m.p. 224°, strongly dextrorotatory, was isolated by Lippman in 1901 from beet molasses. It has no reducing power and is apparently a tetritol.

An interesting contribution to the stereochemistry of the cyclitols has been made by A. L. Patterson.[16]

Theory predicts the existence of 25 isomeric tetritols, 16 pentitols and 9 hexitols; but the number of isomers found in nature is very limited. Patterson has calculated the number of possible isomers, assuming they all contained the grouping

$$\begin{array}{cccccccc} & OH & H & & OH & OH & & \\ — & C & — & C & — & C & — & C & — \\ & H & & OH & H & H & & \end{array}$$

which is contained in the open-chain formula of *d*-glucose.

| | Activity. | Theory. | No. Types Actually Found. |
|---|---|---|---|
| Tetritols . | inact. | 0 | |
| | *d* | 1 | 1 |
| | *l* | 1 | |
| Pentitols . | inact. | 1 | |
| | *d* | 3 | 1 ) not |
| | *l* | 3 | 1 ) antipodes |
| Hexitols . | inact. | 3 | 2 |
| | *d* | 1 | 1 |
| | *l* | 1 | 1 |

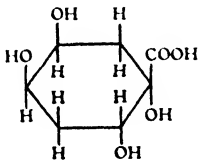
If this restriction is applied, the number of isomeric tetritols is reduced to two, pentitols to seven and hexitols to five, numbers still

in excess of the number of types found in nature. On examination of the configurations of *d*- and *l*-inositol, of *i*-inositol and *d*-quercitol, which have been established, it is seen that these configurations each contain the assumed group derived from *d*-glucose. This is a somewhat striking result which supports the idea that the cyclitols are derived in organisms from the sugars. Further, on this hypothesis scyllitol must possess the first of the two alternative formulæ possible on the chemical evidence, and also the configuration for *l*-quercitol may be narrowed to two alternatives; it will be interesting to see if the Patterson hypothesis is upheld after this prediction, when the configuration of these substances is established.

Needham [17] has obtained good evidence for the synthesis of inositol from glucose in chick and dogfish embryos.

Micheel [18] has accomplished the difficult laboratory transformation from the hexose to the cyclitol series by an extremely roundabout method.

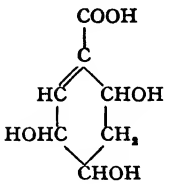
*Quinic Acid*, $C_6H_7(OH)_4COOH$.—Quinic acid is a carboxylic derivative of a tetrahydroxy-cyclohexane occurring in cinchona bark, coffee beans, bilberries and other plants; it is very often found conjugated with caffeic acid as the depside chlorogenic acid. It melts at 162° and is optically active. The investigations of Karrer [19] and of H. O. L. Fischer [20] have proved its structure to be




Many bacteria and mould fungi are able to break down quinic acid, usually to protocatechuic acid, but also sometimes to hydroquinone or pyrocatechin. Some organisms complete the breakdown of the aromatic ring to oxalic acid.

According to Quick [21] the administration of quinic acid, or of prunes which contain it, to man, results in the excretion of benzoic acid conjugated as hippuric acid.

*Shikimic Acid*, $C_6H_6(OH)_3COOH$.—The only natural representative of the trihydroxy-cyclohexanes at present known is shikimic acid, which is found in the fruit of *Illicium religiosum*. It contains a molecule of water less than quinic acid.




These two compounds have been included here because of their systematic relationship to the cyclitols and through these to the sugars; it remains to be established whether they are so derived in nature, or whether they arise by independent synthesis.

## REFERENCES TO CHAPTER XIV.

1. Rhodes and Wiltshire, J. Rubber Research Inst. Malaya, 1932, **3**, 160.
2. Müller, J.C.S., 1912, 2383.
3. Sherrard and Kurth, J.A.C.S., 1929, 3139.
4. Winterstein, Z. Physiol. Chem., 1908, **58**, 118.
5. Contardi, Gazzetta, 1912, **42**, 408.
6. Anderson, J.B.C., 1912, **11**, 47; J.B.C., 1912, **12**, 97.
7. S. Posternak, Compt. rend., 1919, **169**, 138.
8. S. and T. Posternak, Helv. Chim. Acta, 1929, **12**, 1165.
9. Wieland and Wishart, Ber., 1914, **47**, 2082.
10. Müller, J.C.S., 1907, 1767.
11. Sando, J.B.C., **68**, 399.
12. Grötepass, Dissertation, Göttingen, 1931.
13. Daniel and Doran, Biochem. J., 1926, **20**, 1676.
14. Karrer, Helv. Chim. Acta, 1926, **9**, 116.
15. T. Posternak, Helv. Chim. Acta, 1932, **15**, 948.
16. Patterson, Nature, 1931, **127**, 974.
17. Needham, Biochem. J., 1924, **18**,, 891; Biochem. J., 1929, **23**, 319.
18. Micheel, Ann., 1932, **496**, 77.
19. Karrer, Helv. Chim. Acta, 1925, **8**, 195.
20. H. O. L. Fischer, Ber., 1933, **65**, 1009.
21. Quick, J.B.C., 1931, **92**, 65.

# CHAPTER XV.

## THE OLIGOSACCHARIDES.*

*The Disaccharides.*—The disaccharides consist of two monosaccharide residues united through an oxygen atom. They are analogous to the glycosides, a second molecule of sugar serving as the aglucone, and when acted upon by hydrolytic agents—acids or enzymes—they break down by combining with a molecule of water into their constituent simpler hexoses or pentoses which may be either aldoses or ketoses.

The aldehyde group of one of the constituent monosaccharides is substituted in the same manner as is glucose in the methylglucosides: the aldehydic or ketonic group of the second monosaccharide may remain functional or it may disappear. In the former case the disaccharide reduces cupric salts, forms an osazone, and exhibits mutarotation, behaving just as glucose; in the latter all these properties are absent. Accordingly, the disaccharides are classified under two types.

The reducing disaccharides form sparingly soluble phenylosazones, which are difficult to purify, similar to one another, and do not show sharp melting-points as they decompose at the melting-point; moreover, both melting-point and crystalline form are greatly altered by small quantities of impurities. The hydrazones, even those prepared from asymmetrically disubstituted phenylhydrazines, are too soluble, as a rule, to be used for the isolation of disaccharides from aqueous solutions.

Largely as the result of the work of Haworth, most of the uncertainties of disaccharide structure have been removed. The formulæ have undergone revision in the light of the pyranose structure for glucose. In two disaccharides fructose is present in the furanose form; namely, in sucrose and turanose. The carbon atom concerned in the attachment of the second monosaccharide in the reducing sugars of type I has been shown to be either $C_4$ or $C_6$.

* We use this term introduced by Freudenberg to differentiate the di-, tri-, and tetra-saccharides as a group, from the polysaccharides, which are composed of a much greater number of simple units.

The table shows the reducing disaccharides which occur naturally, or are obtained from the naturally occurring polysaccharides.

| Sugar. | Components: Reducing Disaccharides. | Rotatory Power. | | |
|---|---|---|---|---|
| | | α. | Mutarotn. Equilib. | β. |
| Maltose . | Glucose-4-α-glucoside | + *168°* | + 136° | [+ 118°] |
| Cellobiose . | Glucose-4-β-glucoside | + *72°* | + 35° | [+ 16°] |
| Lactose . | Glucose-4-β-galactoside | [+ 90°] | + 55·3° | + 35° |
| Gentiobiose. | Glucose-6-β-glucoside | + 31° | + 9·6° | [− 11°] |
| Melibiose . | Glucose-6-α-galactoside | + *179°* | + 145° | [+ 12·5°] |
| Turanose . | Fructofuranose-6-α-glucoside | | + 75·3° | [+ 22°] |
| Vicianose . | Glucose-6-β-*l*-arabinoside | | + 39·7° | |
| Primeverose | Glucose-6-β-*d*-xyloside | [+ 22·7°] | − 3·4° | |

The ordinary form is indicated by square brackets; calculated values, where only one crystalline form has been isolated, by italics.

### Methylglycosides of the Reducing Disaccharides.

| | $[\alpha]_D$. |
|---|---|
| α-Methyl-gentiobioside . | + 58·5° |
| β-Methyl-gentiobioside . | − 36·0° |
| β-Methyl-maltoside . | + 78·8° |
| β-Methyl-cellobioside . | − 19·0° |
| β-Methyl-lactoside . . | − 38·1° |
| β-Methyl-melibioside . | + 75·0° |

### Fully Methylated Methylglycosides of the Reducing Disaccharides.

| | $[\alpha]_D$. |
|---|---|
| Octamethyl β-maltose . | + 89·5° |
| Octamethyl β-cellobiose . | − 15·9° |
| Octamethyl β-gentiobiose. | − 33·9° |
| Octamethyl β-lactose . | + 5·0° |
| Octamethyl β-melibiose . | + 97·8° |
| Octamethyl turanose | + 106·7° |

### Octacetyl Derivatives of the Reducing Disaccharides.

| | M.p. | $[\alpha]_D$. |
|---|---|---|
| Maltose . . . | α 125° | + 122·7° |
| | β 159° | + 62·6° |
| Gentiobiose . . | α 188° | + 52·3° |
| | β 195° | − 5·6° |
| Cellobiose . . | α 229° | + 42·0° |
| | β 202° | − 14·5° |
| Lactose . . . | α 152° | + 53·6° |
| | β 90° | − 4·7° |
| Melibiose . . | β 177° | + 102·5° |

In naming a reducing disaccharide, the name of the component containing the unsubstituted aldehyde group ends in -ose, whilst the second component is named as a glycoside. The point of attachment of the glycosidic component is indicated by a numeral: the Greek $\alpha$ or $\beta$ indicates the stereochemical nature of the glycosidic link, and if known the configuration of the component with the reducing group free. Thus, maltose is glucose-4-$\alpha$-glucoside, sometimes written 4-$\alpha$-glucosido-glucose. The form of ring is designated 1 : 5 for pyranose and 1 : 4 for furanose sugars. Crystalline $\beta$-maltose written in full is: $\beta$- (1 : 5) glucose-4-$\alpha$-(1 : 5) glucoside, or, avoiding the use of numerals, $\beta$-glucopyranose-4-$\alpha$-glucopyranoside. It is convenient sometimes to indicate the points of attachment by the symbol $\longleftrightarrow$ with appropriate numerals. Thus raffinose is represented as

$$\begin{array}{c} \text{galactose} - \text{glucose} - \text{fructose} \\ 1 \longleftrightarrow 6 \quad 1 \longleftrightarrow 2 \end{array}$$

## Structure of Dissacharides.

The structure of the disaccharides is established by methylation by the Irvine-Haworth procedure. The reducing disaccharides yield a heptamethyl derivative, the non-reducing disaccharides an octamethyl derivative. The methylation is accomplished without change in ring structure or migration of the biose linkage. These fully methylated disaccharides are broken down by acid hydrolysis to two methylated monosaccharides, which can be completely separated and identified.

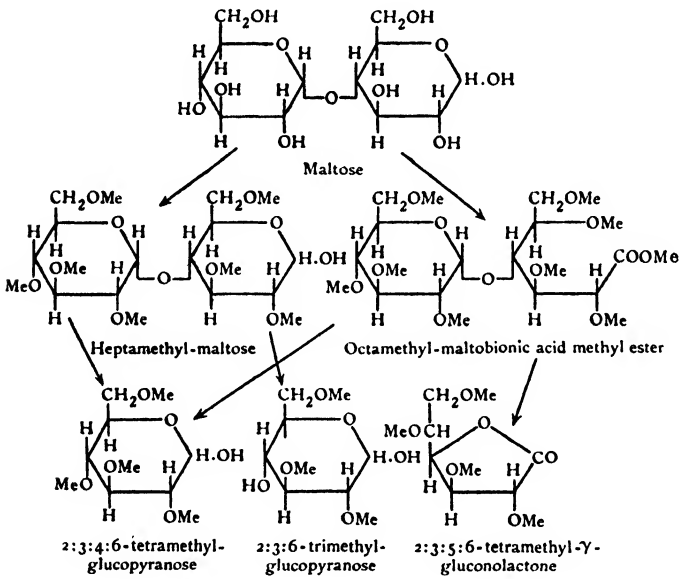
Thus maltose, cellobiose and lactose all give rise to 2 : 3 : 6-trimethyl-glucopyranose and to 2 : 3 : 4 : 6-tetramethylglucose (lactose gives the tetramethylgalactose). The tetramethyl sugar here clearly is the glycosidic component and is attached to either $C_4$ or $C_5$ of the other component in the disaccharide. The fact that the isolated sugar is 2 : 3 : 6-trimethylglucopyranose does not prove that this component is a pyranose in the original disaccharide, since the unstable furanose could revert to the pyranose under the conditions used in isolation.

Gentiobiose and melibiose give rise to 2 : 3 : 4-trimethylglucose and to 2 : 3 : 4 : 6-tetramethylglucose and -galactose respectively. Since a 1 : 6-oxide ring is very improbable and is excluded on other evidence, it may be assumed that the biose linkage occurs at $C_6$ in the component which furnishes the trimethylglucose.

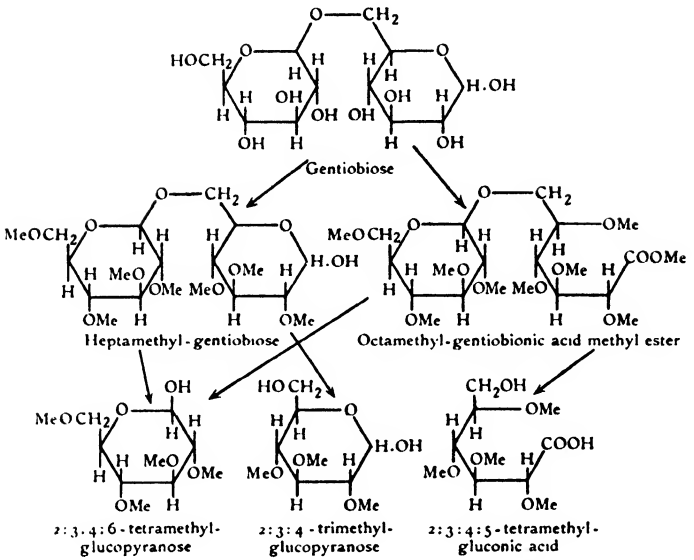
The problem as to which hydroxyl group in the partially methylated monosaccharide is occupied in the biose linkage and which in the oxygen ring can be established with certainty by two methods, one due to Haworth [1] and the second to Zemplen.[2]

The first method consists in the oxidation of the aldehyde group in the disaccharide or its methylated derivative with bromine water to the carboxyl group forming the corresponding bionic acid. Maltobionic acid on complete methylation yields octamethyl-maltobionic acid monomethyl ester, which on hydrolysis yields 2 : 3 : 4 : 6-tetramethylglucose and 2 : 3 : 5 : 6-tetramethylgluconic acid. The position of the biose linkage is clearly fixed as $C_4$. The same proof applies to cellobiose and lactose.

In the same way gentiobiose yields an octamethyl-gentiobionic acid which hydrolyses to 2 : 3 : 4 : 6-tetramethylglucose and 2 : 3 : 4 : 5-tetramethylgluconic acid. The biose linkage in gentiobiose and in melibiose is thus fixed as at $C_6$.




Zemplen's [2] method of determining the position of the biose linkage consists in the stepwise degradation by a modification of Wohl's method. Maltose yields first, glucosido-arabinose and secondly, glucosido-erythrose. The fact that the last does not form an osazone, though it forms a hydrazone, shows that position 2 in the erythrose is blocked and hence that the biose linkage in maltose is at $C_4$.




The stereochemical nature of the glycosidic link in a disaccharide can be ascertained by a knowledge of the action of enzymes, and also from a consideration of the value of the optical rotation.

While the hydroxyl group in the primary alcohol group of $C_6$ is different in reactivity from the other hydroxyl groups of the sugar, there is no special reactivity which characterises the hydroxyl group on $C_4$ to differentiate it from the other secondary alcohol groups.

Some selective influence must apparently be looked for to explain the extremely important rôle played by $C_4$ in the linkage of the units of the most important polysaccharides, starch and cellulose.

At first sight it might appear that the numerous oligosaccharides which have been isolated from plants, and whose constitution has been ascertained, allow no classification but form a haphazard collection of substances. The present knowledge of their structure nevertheless enables them to be included in a remarkably simple scheme.

The linkage of monosaccharides to each other through the reducing group to $C_4$ of a second monosaccharide is only found in those substances such as cellobiose and maltose which are degradation products of very large molecules composed of regular chains of many such units joined together. The rare occurrence of maltose in the free state in plants is indicative of a downgrade process rather than a synthetic one.

The oligosaccharides which contain glucose are all derived from sucrose; by addition of a molecule of glucose or galactose to the glucose portion of this, gentianose and raffinose respectively are produced. Loss of fructose from these yields gentiobiose and melibiose, and finally by oxidation and decarboxylation of the two latter, primeverose and vicianose.

Gentiobiose, primeverose and vicianose are found commonly in glycosides, and a similar function for melibiose will, it may be predicted, probably be discovered.

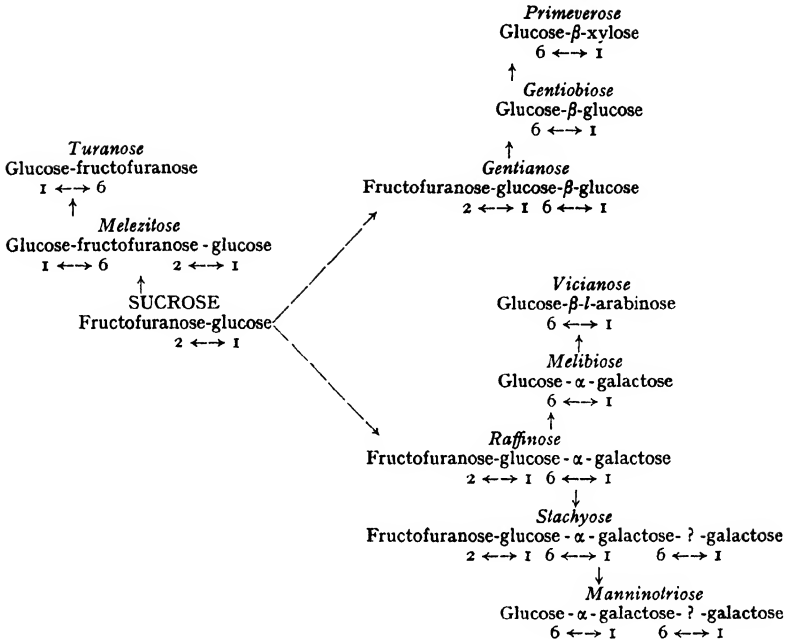
Gentianose and raffinose serve the same purpose as sucrose and can be rapidly mobilised for the needs of the plant.

Raffinose can be elaborated still further to stachyose, and this then by loss of fructose gives a new sugar, manninotriose. It should be said that the relation of raffinose to stachyose is not yet completely established, but that suggested is one interpretation of the evidence, and on biosynthetic grounds is very inviting. It is not improbable that a tetrasaccharide corresponding to stachyose, but based on gentianose, remains to be discovered.

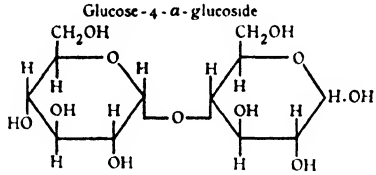
Melezitose derives from sucrose by addition of a molecule of glucose to the fructose portion of the molecule, and gives by loss of the other glucose molecule, turanose. Melezitose has never been established to be present in the cell sap of plants, but is always a product in an exudation caused by sucking insects. It is likely that the investigation of other sugary exudations will result in the finding of new sugars.

Trehalose is particularly a product of lower forms of plant life which are rich in the α-glucosidase known as trehalase, and its occurrence in higher plants is rare, and is usually as an excretion following shock such as a hard frost. Trehalose, αα-diglucose can easily give rise to the glucoxylose of Power and Salway, though this may also have its individual synthesis.

There are several disaccharides and trisaccharides containing rhamnose, frequently associated with galactose, but insufficient is known about this group to attempt a classification. Indeed new work in this group is required.




## Maltose.




Maltose is of chief interest as the disaccharide obtained in the degradation of starch and of glycogen. It has been recorded as present in the free state in a number of plants, but usually it is only a product of secondary origin formed from the starch during the extraction. Definite evidence for the occurrence of maltose in the underground organs of four different plants has been obtained. It occurs in the tubercles of *Umbilicus pendulinus*,[3] in the rhizomes of *Mercurialis perennis*,[4] where the amount increases throughout the summer months to a maximum of 2 per cent. in September; in *Bolbostemma paniculata* [5] in amounts up to 7 per cent. in autumn and in the tubers of *Lathyrus tuberosus*.[6]

It is usually considered that starch is synthesised from sucrose

rather than maltose, and the finding of maltose in the above instances is indicative of the course of degradative rather than synthetic processes.

A sugar was first isolated from the products of hydrolysis of starch by De Saussure in 1819, but it was not until 1847 that this new sugar was further examined by Dubrunfaut and named maltose. This discovery seems to have lapsed into comparative oblivion until the sugar was rediscovered by O'Sullivan in 1872. Maltose is obtained by the action of diastase on starch in almost 80 per cent. yield, the other products being dextrins. It crystallises as the monohydrate, has $[\alpha]_D + 118°$, which increases by mutarotation to an equilibrium of $+ 136°$. The ordinary crystalline form is therefore by definition $\beta$-maltose.

Maltose reduces Fehling's solution, forms a phenylosazone, and shows many of the properties of glucose. It forms an octacetate.

Maltose yields, on oxidation with bromine, an acid containing the same number of carbon atoms, which is termed maltobionic acid ; this is hydrolysed to glucose and gluconic acid by mineral acids. Maltose combines with hydrogen cyanide, forming a compound which on hydrolysis gives maltose carboxylic acid, and is hydrolysed by mineral acids to glucose and glucoheptonic acid.

The enzymes, diastase, invertase, lactase and emulsin, are without action on maltose, maltase alone of all the known enzymes being able to effect hydrolysis. Maltose is fermented only by those yeasts which contain maltase, and then not until hydrolysis has been brought about by the enzyme. Because of the behaviour of maltose towards maltase, it is considered to be a glucose-$\alpha$-glucoside, and in confirmation, $\alpha$-glucose has been proved to be formed initially on hydrolysis.

Maltose forms a glycoside analogous to methylglucoside, but the direct condensation with methyl alcohol in presence of acid is not possible, as the disaccharide becomes hydrolysed during the operation. $\beta$-Methyl maltoside is prepared from acetochloro maltose, obtained by the action of hydrogen chloride on maltose octacetate : acetochloro maltose reacts with methyl alcohol in presence of silver carbonate, forming heptacetyl methylmaltoside, which is converted into methylmaltoside on hydrolysis with baryta. The behaviour of this maltoside towards enzymes is instructive. Maltase hydrolyses it at the $\alpha$-junction, forming glucose and $\beta$-methylglucoside ; emulsin attacks only the $\beta$-junction, forming maltose and methyl alcohol. The maltoside is accordingly $\beta$-methyl glucose-$\alpha$-glucoside.

As a result of methylation studies [7] the structure of maltose is firmly established as glucose-4-$\alpha$-glucoside.

Freudenberg and Kuhn [8] have studied the kinetics of the acetolysis of starch, and the results can best be reconciled with the view that starch consists of a regular chain of α-glucose units, each unit united by its reducing group to $C_4$ of the adjoining unit. β-Linkages and furanose rings are excluded.

Further, it is possible to methylate the products of mild acetolysis and to separate the fully methylated maltose, maltotriose and maltotetraose from the mixture.[9] Starch therefore yields, like cellulose, a series of oligosaccharides on mild hydrolysis.

## Isomaltose.

Isomaltose is a name used to characterise a number of different disaccharide mixtures which have in common the ability to form osazones and are non-fermentable, but differ widely in other respects. The vague way in which the term has been used is unfortunate.

The name isomaltose was first given by Fischer [10] to the disaccharide obtained by him by the condensing action of strong acids at room temperature on glucose. It was characterised only by means of the phenylosazone, and was obtained by alcohol-ether precipitations from the residue after destroying the fermentable sugars with yeast.

Scheibler and Mittelmeier [11] obtained a substance with the same melting-point as Fischer's isomaltosazone by the action of 2·5 per cent. sulphuric acid on a 10 per cent. glucose solution at 100°.

A quantitative study of the action of hydrochloric acid of much lower strength (0·7 normal) than used by Fischer on glucose has been made by Harrison.[12] He isolated unfermentable *iso*maltose, and showed that in 52 per cent. glucose solution the final ratio of *iso*maltose to glucose is 2 : 3. Davis finds that synthesis of *iso*maltose takes place in a 1 per cent. solution of glucose in fuming hydrochloric acid (40 per cent. acid).

Products similar to *iso*maltose have been repeatedly described as obtained in the hydrolysis of starch, e.g. gallisin. *Iso*maltose is probably identical with the disaccharide obtained by Croft Hill [13] by the synthetic action of maltase on glucose, which he termed revertose. E. F. Armstrong [14] showed that isomaltose was hydrolysed by emulsin, but not by invertase or maltase, and considered the isomaltose obtained by means of acids or enzymes to be the same.

*Iso*maltose as described by Lintner, who first obtained it from starch, has $[\alpha]_D + 139°$: the phenylosazone which was readily soluble in hot water has m.p. 152° $[\alpha]_D + 61°$. Later observers

considered it a mixture of maltose and dextrin, but a similar unfermentable fraction was obtained by Ling and Baker in 1897.

According to Ling and Nanji the most convenient way of preparing it is to let precipitated malt diatase act on crude amylopectin or on α-hexa-amylose at 50°, remove the glucose formed by fermentation and purify the syrup by treatment with alcohol in the usual manner. The white hygroscopic powder obtained cannot be induced to crystallise: it has a sweeter taste than maltose. The rotatory power $[\alpha]_D$ + 140° and the reducing power are as stated by Lintner.

Haworth [15] has stated that a sample of Ling and Nanji's *iso*-maltose was converted by him on acetylation into octacetylmaltose.

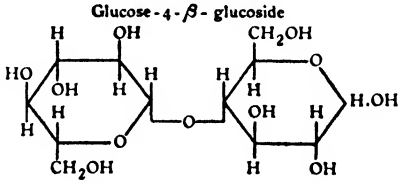
It seems to be established that gentiobiose is also a component of *iso*maltoses, which are obviously mixtures. Berlin [16] has obtained gentiobiose octacetate from the unfermentable residue of the action of hydrochloric acid on glucose. Berlin also states that during acid hydrolysis of starch under certain conditions 5·7 per cent. is converted into gentiobiose: there is here, however, a complication in that starch may contain some preformed gentiobiose apart from that which is synthesised.

Georg and Pictet [17] state that the action of hydrochloric acid at 0° on glucose yields 36 per cent. of *iso*maltose, but only 1 per cent. of gentiobiose. They give the following properties for *iso*maltose: m.p. 145°, $[\alpha]_D$ + 94·5°, osazone, m.p. 160°, $[\alpha]_D$ + 23·1°.

It is unprofitable in the present state of knowledge to discuss the isomaltose question at length. It is best to regard the term as a designation for a mixture of various known and unknown diglucoses.

## Cellobiose.

Glucose-4-β-glucoside




Franchimont [18] obtained on acetolysis of cellulose a crystalline acetate of a new sugar, but it was Skraup [19] who first prepared the free disaccharide known as cellobiose. This isolation of an intermediate substance in the breakdown of cellulose to glucose was the beginning of the knowledge of the detailed structure of cellulose. Just as starch contains maltose residues, so cellulose contains cellobiose residues.

The relation of cellobiose to maltose is just the same as that of β-methyl to α-methylglucoside, and it is a striking fact that such a superficially small difference has as its consequence the very great difference between the two extremely important polysaccharides, cellulose and starch. The constitution of cellobiose has been definitely established by Haworth and Hirst [20] and Haworth, Long and Plant ; [21] also Zemplen.[2]

Cellobiose is not attacked by invertase or maltase ; it is hydrolysed by emulsin and therefore has a β-glycosidic structure. An enzyme from the snail breaks down β-methylcellobioside into glucose and β-methylglucoside, the hydrolysis of this latter substance being sufficiently slow to permit of its isolation.[22]

β-Cellobiose has m.p. 225°, $[\alpha]_D$ + 16°, which increases by mutarotation to + 35° ; the α-form has a calculated $[\alpha]_D$ + 72°. It has a faintly sweet taste and is much less soluble than sucrose. It reduces Fehling's solution, and forms a phenylosazone and an osazone. It forms acetochloro and acetobromo derivatives, and in chemical properties is very similar to maltose and lactose.

The α-octacetate prepared by acetylation of the sugar in presence of zinc chloride or sulphuric acid has m.p. 229°, $[\alpha]_D$ + 42°. The β-octacetate is obtained on acetylation in presence of sodium acetate and has m.p. 202°, $[\alpha]_D$ − 14·5°. It is transformed into the α-isomeride on heating with acetic anhydride in presence of sulphuric acid.

Cellobiose octacetate is changed by aluminium chloride into the acetate of an isomeric hexose, celtrobiose,[24] whose constitution has not been determined.

Cellobiose can be converted into the unsaturated reduction product cellobial, analogous to glucal, which on treatment with perbenzoic acid yields crystalline 4-β-glucosido-mannose, $[\alpha]_D$ + 15°. This is slowly hydrolysed by emulsin.[23]

Zemplen degraded cellobiose to 3-β-glucosido-arabinose in 80 per cent. yield.

Cellobiose has been synthesised by Freudenberg and Nagai,[25] by the condensation of acetobromoglucose with lævoglucosan, to give an intermediate product which was converted in 50 per cent. sulphuric acid into cellobiose tetracetate. Octamethyl cellobiose was obtained by Freudenberg, Anderson and Go [26] by condensing 2 : 3 : 6-trimethylglucose β-methylglucoside with 2 : 3 : 4 : 6-tetramethyl-glucose 1-chlorohydrin.

When cellulose is treated with acetic anhydride containing a little sulphuric acid it is acetolysed, that is, both acetylated and hydrolysed.

The study of the kinetics of acetolysis, and of the products formed, has been of great value in elucidating the structure of cellulose.[27]

Cellobiose acetate is the main product, and can be isolated in amount corresponding to a 40 per cent. yield of cellobiose; by correcting for losses, it can be calculated that the total amount of cellobiose produced is 60 per cent.

Cellobiose is also a product of acetolysis of lichenin and of tunicin.

The successive degradation of cellulose yields first dextrins, then smaller fragments, among which can be identified cellobiose, cellotriose,[28] cellotetraose and a cellohexaose.[29]

The two latter products are more easily isolated from the products of hydrolysis of cellulose with hydrochloric acid.

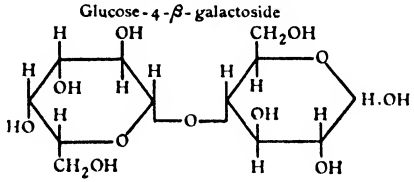
Freudenberg [30, 9] was able to methylate the acetolysis mixture, and separate out the fully methylated cellotriose and cellotetraose by distillation.

The product obtained by Bertrand known as procellose is identical when purified, with cellotriose, and the *iso*cellobiose of some workers is a mixture of cellobiose with cellotriose.[31]

Freudenberg and Nagai [32] have synthesised the fully methylated hendecamethyl cellotriose by a method which shows cellotriose to be 4-cellobiosido-glucose.

The cellodextrins can be partially separated into fractions of different mean molecular weight, and the size of the molecule can be estimated either by iodine titration,[33] which determines the number of free aldehyde groups, or by determining the proportion of tetramethylglucose obtained from hydrolysis of the fully methylated cellodextrins.[34] Both methods agree in finding that the cellodextrins range in size from chains of 10 to chains of 30 $\beta$-glucose units.

## Lactose.




Lactose or milk sugar, discovered in 1615 by Fabriccio Bartoletti, in Bologna, occurs in the milk of all animals, but has not been encountered in the vegetable kingdom. Human milk contains 6 per cent. and cow's milk about 4 per cent. of lactose. It is manufactured by

evaporation of whey, purified by recrystallisation, and obtained as a white crystalline powder. Mineral acids hydrolyse it to glucose and galactose; it exhibits mutarotation, reduces Fehling's solution, and forms a phenylosazone soluble in boiling water. Like glucose it gives rise to two series of isomeric derivatives, e.g. octacetates, acetohalogenolactoses and methyl lactosides. Two modifications of the sugar itself exist corresponding to the α- and β-isomerides.

The milk sugar of commerce is α-lactose, $[\alpha]_D + 90°$. The β-form has + 35° and the equilibrated mixture + 55·3°.

Lactose was the second sugar to have its mutarotation studied by Dubrunfaut, and Tanret early established the existence of two forms of it. There is an extensive literature on the subject. Lactose is a glucose-galactoside, since on oxidation with bromine, lactobionic acid is formed, and this when hydrolysed by mineral acids gives gluconic acid and galactose, proving that the reducing group is in the glucose part of the molecule.

The formula of lactose has been definitely established by Haworth;[35] it shares a common type structure with maltose and cellobiose, differing from cellobiose only in respect of the orientation of groups attached to one carbon atom; yet the rôle of the two substances in nature is so entirely different. When the calcium salt of lactobionic acid is oxidised by Fenton's method, a *d*-arabinose-3-galactoside is obtained, which is of interest as an example of a synthetic disaccharide containing both hexose and pentose sugars.[36] The formation of this galactosido-arabinose affords additional proof that lactose is a galactoside. *d*-Arabino-3-galactoside has been also prepared by Zemplen[37] from the oxime of lactose. It has $[\alpha]_D - 58°$, and is said to differ from Ruff's product.

A *neo*lactose which is 4-galactosido-*d*-altrose is obtained to the extent of 30 per cent., when anhydrous aluminium chloride interacts with lactose octacetate;[38] a Walden inversion occurs, and the groups on both $C_2$ and $C_3$ in the glucose component are inverted.

Lactulose = fructose-4-β-galactoside is obtained through a Lobry de Bruyn reaction by the action of calcium hydroxide on lactose.[39]

Lactose is hydrolysed by a specific enzyme, lactase, found in a few yeasts (or, more correctly, torulæ), and in some kefir preparations, and in the enzyme (crude emulsin) contained in an aqueous extract of almonds. Lactase is a β-galactosidase, hydrolysing all β-galactosides, and according to Helferich is identical with the β-glucosidase of emulsin. Lactose is not hydrolysed by maltase, invertase, diastase, nor by any of the enzymes of dried brewers' yeast. Only those

yeasts (torulae) which contain lactase are capable of fermenting milk sugar. Lactose is particularly prone to undergo lactic and butyric acid fermentations.

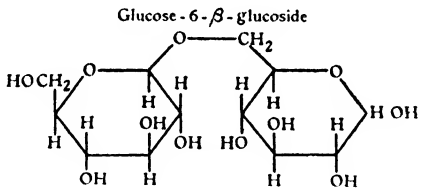
The galactosido-arabinose obtained by the degradation of lactose is also hydrolysed by crude emulsin. Further degradation yields *d*-erythrose galactoside, which is not hydrolysed by emulsin.

*Iso*lactose is the name given to a disaccharide obtained by Fischer and Armstrong [40] by the synthetical action of the enzyme kefir lactase on a concentrated solution of equal parts of glucose and galactose, and isolated in the form of the phenylosazone. It has not been further studied and is probably a mixture.

Two new disaccharides related to milk sugar in giving glucose and galactose on hydrolysis have been isolated from human milk serum by Polonovski and Lespagnol.[41] They are gynolactose, $[\alpha]_D$ $-27°$, which has no reducing properties, and allolactose, $[\alpha]_D$ $+20°$, which has such properties; they were separated by fractional crystallisation.

Allolactose is hydrolysed by emulsin, and its properties and those of its derivatives are similar to those of the synthetic glucose-6-$\beta$-galactoside of Helferich and Rauch.[42]

## Gentiobiose.



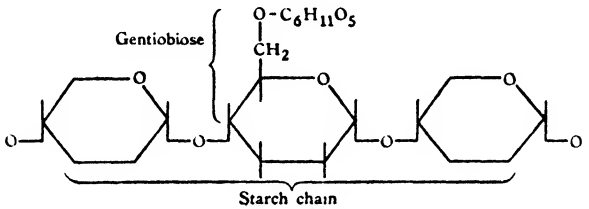

Gentiobiose is a constituent of a number of glycosides, notably crocin and amygdalin and members of the anthocyanins, and it is probable that it will be found to be of wide distribution. It can be prepared from amygdalin by catalytic hydrogenation.[43]

Gentiobiose is also a component of the trisaccharide gentianose, present in the roots of various *gentian* species. The trisaccharide is partially hydrolysed by invertase or by dilute acids to fructose and gentiobiose. A method of preparation due to Haworth and Wylam [44] yields 40 grams of the octacetate from a kilogram of powdered gentian root. Saponification of the acetate regenerates gentiobiose itself.

Gentiobiose is said to be formed in small amounts on hydrolysis of the $\beta$-amylose fraction of maize starch, under conditions which

render it improbable that it is synthesised by the action of the acid used for hydrolysis upon the glucose liberated in the hydrolysis.

The small yield may be due to impurity, but if the gentiobiose is a real constituent of the starch its production does not contravert the theory of a continuous chain of α- glucose units. A glucose unit could be attached by a β-linkage to one of the free primary hydroxyl groups in the chain, thus forming a branched chain containing the gentiobiose molecule. While the existence of such structures is purely speculative at present, their existence in both starch and cellulose is quite plausible ; it may be noted that their existence would invalidate the Haworth method of determining chain length, since such structures would also yield tetramethylglucose.



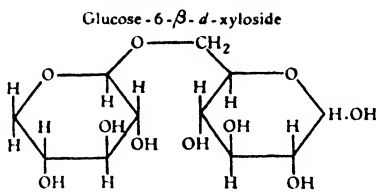

Gentiobiose crystallises as the β-form, $[\alpha]_D - 11°$, which mutarotates to $+ 9{\cdot}6°$, the α-form having $[\alpha]_D + 31°$. The phenylosazone melts at 162°-167°.

Since gentiobiose is hydrolysed by emulsin the biose linkage is β-glucosidic.

An enzymic synthesis of gentiobiose was achieved by Bourquelot,[45] by the action of emulsin on a concentrated glucose solution. Zemplen has confirmed this observation.

The chemical synthesis of gentiobiose has been achieved by Helferich, Klein and Schäfer,[46] and the structure established by Haworth.[47]

## Primeverose.

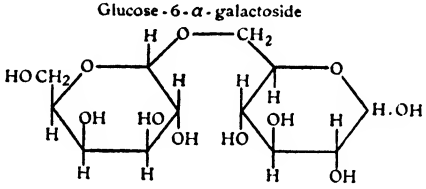



Primeverose is only found as a constituent of glycosides, viz. in gaultherin or monotropin, present in the *Betulaceæ* and the *Ericaceæ*, in rhamnicosin, present in *Rhamnus catharticus*, in primeverin and

primulaverin from *Primula officinalis*, in genticaulin, the glycoside of the rhizome of the large yellow gentian, *Gentiana lutea*, and in other gentian species. Its preparation from primeverin is described by Goris, Mascré and Vischniac.[48] The crystals melt at 208° and the specific rotation falls from + 22·7° to — 3·4° on standing. It has been synthesised by Helferich and Rauch.[49] From its relation to gentiobiose, it is possible that it is formed in the plant from this sugar via the uronic acid in the same way as xylose originates from glucose. It is significant that gentiobiose as a component of the trisaccharide gentianose, is also present in the roots of the various species of gentian which contain genticaulin.

## Melibiose.

Glucose-6-α-galactoside




Melibiose, together with fructose, is obtained from the trisaccharide raffinose by hydrolysis with dilute acids or invertase. It crystallises with difficulty and is very soluble in water, and it is advisable to remove the fructose from the products of hydrolysis of raffinose by fermentation with a top yeast before attempting to isolate it.

Hudson obtained as much as 200 grams from 500 grams of raffinose by fermenting with bakers' yeast in 10 per cent. solution for 36-48 hours. Harding [50] has obtained improved yields by using glacial acetic acid for the final recrystallisation. Melibiose exhibits mutarotation, the α-form having $[\alpha]_D$ + 197°, the β-form + 125°, and the equilibrium mixture + 143°. When hydrolysed with strong acids melibiose yields glucose and galactose. On reduction with sodium amalgam, an alcohol, melibiitol, is formed which, when hydrolysed, is converted into mannitol and galactose.

Melibiose forms a phenylosazone and an osone, which hydrolyses to galactose and glucosone: and a series of α- and β-derivatives in the same manner as other reducing sugars.

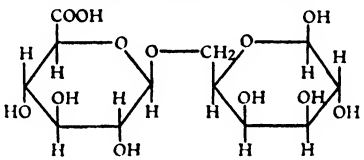
Melibiose octacetate is synthesised by the condensation of acetobromogalactose with 1 : 2 : 3 : 4-tetracetylglucose in presence of quinoline, the conditions under which α-galactosides are produced.[51] If the condensation is carried out with silver oxide the isomeric

β-derivative is obtained, and thus Helferich and Rauch [52] obtained glucose-6-β-galactoside, which may be identical with allolactose from human milk serum. Pictet and Vogel [53] make a claim to have synthesised melibiose from diglucosan and digalactosan.

Melibiose is slowly hydrolysed by crude emulsin, more rapidly by an enzyme contained in bottom fermentation, but not in top fermentation yeasts: this enzyme is appropriately termed melibiase, and is an α-galactosidase, splitting α-galactosides. Melibiose is not attacked by maltase, invertase or lactase. It affords a chemical means of distinguishing between top and bottom fermentation yeasts.

The behaviour towards crude emulsin led at one time to the deduction that it was a β-galactoside, but the results of synthesis and the optical properties of its derivatives [47] determine it as a derivative of α-galactose.

Galactose-6-glucuronic acid.

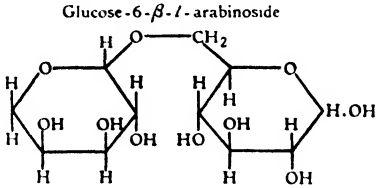


An aldobionic acid, $[\alpha]_D$ — 2·0°, obtained as a hydrolysis product of gum-arabic, is assigned the above structure by Haworth.[54] Methylation gives a heptamethyl aldobionic ester which on hydrolysis yields 2 : 3 : 4-trimethylgalactose and 2 : 3 : 4-trimethylglucuronic acid. The β-linkage at the biose junction is not definitely established.

Similar aldobionic acids composed of glucose and glucuronic acid are components of the polysaccharides of type III. pneumococcus and Friedländer's bacillus; their structure has yet to be worked out. The aldobionic acid from flax-seed mucilage yields rhamnose and galacturonic acid on hydrolysis.

## Vicianose.

Glucose-6-β-*l*-arabinoside



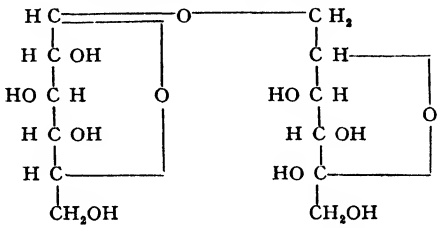
Vicianose does not occur in the free state, but it has been found as a constituent of several glycosides, notably gein, from *Geum urbanum*,

vicianin in *Vicia angustifolia*, and violutin in *Viola cornuta*. The preparation from vicianin is described by Bertrand and Weisweiler.[55] The colourless needles melt at 210°, are soluble in water, and have a final rotation $[\alpha]_D^{20°} + 39{\cdot}7°$. It is not fermented, but is hydrolysed by the α-galactosidase of emulsin to *d*-glucose and *l*-arabinose.

Its synthesis has been accomplished by Helferich and Bredereck.[51]

## Turanose.

Fructopyranose-6-α-glucoside.



Turanose was discovered by Alekhine [56] in 1889 as a product, together with glucose, of the partial hydrolysis of the trisaccharide melezitose, with weak acids. He stated that it yielded two molecules of glucose on further hydrolysis, but Tanret [57] subsequently showed that an equimolecular mixture of glucose and fructose is produced. Turanose is hydrolysed only with difficulty by acids. Until turanose was obtained crystalline by Hudson and Pacsu,[58] its structure was in part based on the result of experiments starting with melezitose.

When crystals of turanose are once obtained from hydrolysed melezitose they cause rapid crystallisation in syrupy preparations.

The pure sugar has m.p. 157°. It is not hygroscopic, and exhibits mutarotation from $[\alpha]_D + 22°$ initially to a final value of $+ 75{\cdot}3°$.

It is the only known reducing disaccharide containing fructose, and is isomeric with sucrose.

It is hydrolysed fairly rapidly by α-glucosidase,[59] and is therefore classed as an α-glucoside. Invertase is without action on it, as are also emulsin and rhamnase.[60]

The products of methylation [61] are 2 : 3 : 4 : 6-tetramethylglucose and 1 : 3 : 4-trimethylfructose, suggesting the conclusion that turanose is fructofuranose-6-α-glucoside.

The isolation of the trimethylfructose, however, does not prove the furanose structure of the fructose component, an alternative being fructopyranose-5-α-glucoside. Pacsu [62] has attempted to settle the question, citing as evidence the fact that turanose readily yields

a tri-triphenylmethyl derivative, indicating the presence of three primary alcoholic groups, which according to him would only result from the fructopyranose formula. The evidence is not, however, conclusive, and it might well be that the third triphenylmethyl group substitutes some group other than a primary alcohol group such as the hydroxyl of the ketone carbonyl.

Other evidence speaks definitely for the furanoside formula. It is very probable that in melezitose fructofuranose exists, and hence also in turanose. The evidence is discussed under the trisaccharide melezitose.

## Strophanthobiose.

*Cymarose-glucoside.*

This is the sugar of Kombé Strophanthin.[63] It is hydrolysed to glucose and cymarose, which is regarded as a methyl-ether of digitoxose. The older statement that the disaccharide consists of mannose and rhamnose is incorrect.

## Rutinose.

*Glucose-l-rhamnoside.*

Rutinose is the sugar of the glycosides rutin and datiscin[64] and is probably of wide distribution. It has $[\alpha]_D$ + 3·2° in water, — 10° in alcohol.

## Non-Reducing Disaccharides.

In the non-reducing disaccharides the junction must obviously be through the reducing carbons, viz. $C_1$ in glucose, $C_2$ in fructose.

| Sugar. | Components. No Reducing Properties. | Rotatory Power. $[\alpha]_D$. |
|---|---|---|
| Sucrose . | 1-α-glucoside-2-β-fructofuranose | + 66·5° |
| Trehalose . | αα-diglucose | + 197° |
| Glucoxylose . | 1-glucose-1-xylose | — 36·5° |

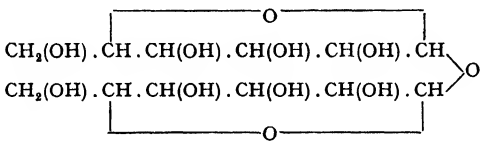
| | Octacetate. | | Octamethyl Derivative. |
|---|---|---|---|
| | m.p. | $[\alpha]_D$. | $[\alpha]_D$. |
| Trehalose . | 97° | + 162·3° | + 199·8° |
| Sucrose . | 69° | + 59·6° | + 69·3° |

As either sugar can be oriented as $\alpha$ or $\beta$, four or three isomerides are possible in theory, according as the components are both different or both the same. All three pyranose forms of the diglucose trehalose have been synthesised, but only one form of trehalose and one form of sucrose occur naturally.

## Trehalose.

*αα-Diglucose.*

Trehalose, which occurs widely distributed in fungi, is also found in bacteria, e.g. in the timothy-grass bacillus ; it is composed of two glucose molecules fused togther, so that both aldehydic functions have disappeared :—



This structure is indicated by the fact that it does not reduce Fehling's solution or form a phenylosazone or exhibit mutarotation. It is not affected by the enzymes maltase, invertase, emulsin or diastase, but is hydrolysed by a special enzyme named trehalase, which is contained in certain fungi and in many species of yeast : this enzyme appears to be different from the $\alpha$-glucosidase, maltase. Trehalase is conveniently obtained from *Aspergillus niger*. Trehalose is only hydrolysed by acids with considerable difficulty, and contrasts markedly in this respect with sucrose.

Apparently trehalose replaces sucrose in those plants (fungi) which contain no chlorophyll and do not manufacture starch. The quantity of trehalose is a maximum just before the formation of spores. When the fungi are picked the trehalose is rapidly converted into mannitol, being hydrolysed first by trehalase to glucose, which is in some way then reduced. To obtain trehalose, the fungi must be extracted with boiling solvents, so as to destroy the enzyme, within two or three hours after gathering.

Harding [65] prepares trehalose from Trehala manna with a yield of 20-25 per cent. by extraction with 75 per cent. alcohol : the extracts when clarified and evaporated, crystallise on the addition of 95 per cent. alcohol.

The best source for its preparation is *Selaginella lepidophylla* (the resurrection plant), obtainable in large quantities in the arid

south-west of N. America; this contains 2 per cent. of the sugar which is readily obtained crystalline.

Trehalose is also a constituent of sea-weeds. Some of the *Florideæ* contain up to 10 per cent. of the dried material.

Trehalose crystallises in lustrous rhombic prisms $[\alpha]_D + 197°$. Its constitution as a pyranose derivative has been confirmed by Bredereck.[66] The natural sugar is the $\alpha\alpha$-form; the two isomerides have been prepared by synthetical methods.

Fischer and Delbrück [67] obtained *iso*trehalose, the $\beta\beta$-form, by condensing tetracetyl glucosidyl bromide in the presence of silver carbonate. $\alpha\beta$ or *neo*-trehalose was prepared by the union of triacetylglucose 1 : 2 anhydride with tetracetylglucose in benzene solution by Haworth and Hickinbottom.[68] Their optical properties are

| | | |
|---|---|---|
| Trehalose | + 178° (hydrate), | + 197° (anhydrous) |
| *Neo*trehalose | + 95° (hydrate), | + 100° (anhydrous) |
| *Iso*trehalose | | − 39° (anhydrous) |

Another $\alpha\beta$-form is claimed to have been prepared by Vogel and Kurnicka [69] with $[\alpha]_D + 67°$.

A most interesting fact relating to trehalose is the formation of its phosphoric ester, when either glucose or fructose are fermented in presence of phosphate with dried yeast.[70]

## Glucoxylose.

This is a non-reducing sugar of the trehalose type, present in the leaves and twigs of *Daviesia latifolia* as a dibenzoyl derivative.[71] It has $[\alpha]_D - 36{\cdot}5°$.

A carbohydrate, $C_3H_8O_4$, m.p. 148°, has been isolated in small quantities from cabbage leaves by Buston and Schryver.[72] It is sweet, non-reducing, and is not hydrolysed by acids. It may be thought of as formed from glycollic aldehyde and formaldehyde

$$CH_2OH . CHOH . O . CH_2OH$$

## Sucrose.

Sucrose, saccharose, cane or beet sugar, industrially the most important of the sugars, is widely distributed in the vegetable kingdom, where it functions almost entirely as a reserve material. In contrast to most of the sugars, it crystallises exceedingly readily; this is almost certainly due to the fact that a single substance and not a mixture of mutarotating isomerides is present in solution.

It is very soluble in water, and has a much sweeter taste than glucose, but is not so sweet as invert sugar.

Sucrose is the cheapest energy-producing foodstuff: it yields 1·815 kilocalories per pound, equivalent with sugar at 2½d. per lb. to 8·71 kilocalories per shilling. The Royal Society (War) Committee estimated that before the war white sugar accounted for 12 per cent. of the energy value of the total food supply of the United Kingdom.

Commercial sugar is obtained from the sugar cane and the sugar beet.* The former is propagated from cuttings, the latter is grown from seed, principally in the North Temperate Zone. The white sugars from the two sources are indistinguishable from each other.

The native country of the sugar cane is unknown, but it is known that it has been cultivated in eastern tropical Asia from great antiquity and spread westward and eastward, reaching the New World early in the sixteenth century and the West Indies in 1641. Markgraf in 1747 found sugar in the beet, thereby pointing the way to the beet-sugar industry, which did not commence, however, until 1801. The cane-sugar industry now accounts for two-thirds of the world's total annual production of 29 million tons. Under cultivation the average yield per acre and the sugar content of the beets has steadily increased, and that of the winning crops in 1929 in this country was 15·6 tons per acre, with a sugar content of 17·6 per cent. The amount of sugar produced from the cane per acre greatly exceeds this, being as high as 6·8 tons in Java, whilst a new variety has given 10 tons in Barbados. The methods of growing the cane, extracting the sugar, refining and transporting it in bulk continue to be improved.

Many of the industrial uses of sucrose are too well known to need mention. A new use is for increasing the strength of lime sand mortar.[73] An increase of 60 per cent. in tensile strength is obtained, with the addition of sucrose amounting to 6 per cent. of the quicklime.

Efforts are now being made to make use also of its derivatives. The octacetate, which can be obtained commercially in 50-lb. lots with a 90-95 per cent. yield by the use of acetic anhydride, using sodium acetate as catalyst, has been employed in the treatment of paper, and in lacquers as a plasticiser. Of other sucrose products, the esters of levulinic acid have been found to be rapid solvents of

* For a full account of the Beet Sugar Industry at home and abroad, see Ministry of Agriculture Report, Economic Series No. 27, 1931; also The World's Sugar Industry by Lewis Eynon, Institute of Chemistry Streatfield Memorial Lecture, 1929.

nitrocellulose. Levulinic acid, or β-acetyl propionic acid, can be obtained in 42 per cent. yield by autoclaving sucrose with 6·5 per cent. hydrochloric acid for one hour at about 160° C.[74]

Naturally cane sugar early engaged the attention of the chemist, and the first experiments to determine its empirical formula date from Lavoisier, though this was only definitely determined by Liebig in 1831.

Cane sugar neither reduces Fehling's solution nor exhibits mutarotation, and it lacks both aldehydic and ketonic properties; mineral acids hydrolyse it to glucose and fructose with great ease. Sucrose is dextrorotatory but, since fructose is more lævorotatory than glucose is dextrorotatory, the products of hydrolysis rotate polarised light in the opposite sense to cane sugar. The change in rotation is from + 66·5 to − 20°: the process is hence termed inversion, and the product invert sugar. The same change is brought about by an enzyme present in yeasts, moulds, in many plants, also in bees and other animals, and termed invertase or sucrase. Cane sugar is fermented by yeasts only after previous inversion with the invertase of the yeast. Accordingly, it is not fermented by yeasts which do not contain invertase, e.g. *S. octosporus*.

Sucrose forms no compounds with phenylhydrazine, and is stable towards alkali: this is in marked contrast to the behaviour of the aldoses and ketoses. Sucrose will withstand heating in alkaline solution at temperatures up to 130° without appreciable decomposition. It also does not give rise to glycosidic derivatives. It contains eight hydroxyl groups, as evidenced by the formation of an octacetate and an octamethyl derivative, but gives rise to one form only of these derivatives.

It forms saccharates, $C_{12}H_{21}O_{11}M$, with sodium and potassium hydroxides and more complex saccharates with lime, strontia and baryta, which are utilised in its industrial purification.

When sucrose is oxidised by nitric acid, oxalic acid is formed; indeed the reaction is of considerable historic interest as oxalic acid was first isolated in this way about 1776 by Bergman, though Scheele had identified the acid shortly before. Chattaway and Harris have found that mesoxalic acid is also a considerable product of the reaction when the oxidation is violent.

The application to sucrose of an entirely satisfactory constitutional formula has long been a matter of difficulty and controversy. It will serve no purpose here to indicate the various proposals even in historical sequence, as it is highly probable that the formulation

as α-glucopyranose-fructofuranose proposed by Haworth affords a satisfactory solution of a very difficult problem.

Sucrose is at one and the same time a glucoside and a fructoside in which the hexose units are so joined as to mask both aldehyde and ketone groups and give a product which is indifferent towards aldehydic reagents.

From invert sugar ordinary glucose and fructose may be obtained crystalline, yet the first product of hydrolysis is the labile γ-fructose, or fructofuranose, which reverts to fructopyranose.

Sucrose → glucopyranose + fructofuranose
↓
fructopyranose.

When sucrose is heated in absolute methyl alcohol at 130°, it undergoes methanolysis,[75] the products being glucopyranose and α-methylfructofuranoside, which cannot revert to the pyranoside form.

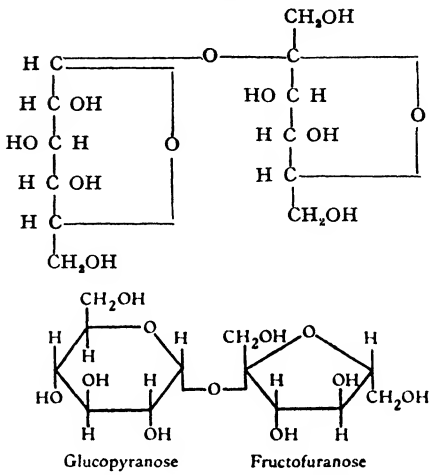
As first pointed out by Haworth and Law,[76] on hydrolysis of sucrose the primary products α-glucopyranose and fructofuranose give rise when the solution attains equilibrium, to a complex mixture of the α- and β-stereoisomers of the pyranose and furanose forms of glucose and fructose. Glucopyranose and fructopyranose forms preponderate in the final equilibrium, but all the forms of glucose and fructose which occur in their simple solutions, even in minute amounts, are also present.

SUCROSE
↓
α-fructopyranose
α- and β-glucofuranose ⇌ α-glucopyranose + ? fructofuranose ⇌ ↓ ↑
etc. ↓ ↑ β-fructopyranose
β-glucopyranose

A great many changes are therefore taking place at the same time when sucrose is hydrolysed, and it is only the fact that the addition of a drop of alkali produces an instant equilibrium which has enabled the hydrolysis to be followed with such accuracy with the polarimeter.

Haworth and Law first showed that octamethyl sucrose did not undergo inversion on hydrolysis, the rotation falling from + 66·5° to + 56·5°, whereas a mixture of methylated normal forms of glucose and fructose would have a rotation of − 18°. The products of hydrolysis were identified as 2 : 3 : 4 : 6-tetramethyl glucose and 1 : 3 : 4 : 6-tetramethyl fructose. Sucrose therefore contains glucopyranose and fructofuranose.

Sucrose.



Glucopyranose Fructofuranose

The proof of structure depends upon the assumption that sucrose methylates without changes of ring structure: the assumption is probably entirely justifiable. Irvine and Stiller [77] consider that there is some evidence for instability of ring structure during methylation.

All sugars containing furanose rings have their glycosidic derivatives hydrolysed at about one thousand times the rate of the corresponding pyranose derivatives.[78]

Similarly, those oligosaccharides which contain fructofuranose are as sensitive as sucrose to dilute acids. Raffinose, gentianose and stachyose are readily hydrolysed by weak acids as well as by invertase; melezitose is also hydrolysed by weak acids but not by invertase, because substitution of the fructose by a second molecule of glucose makes it impossible for the enzyme to act.

The glucose constituent is regarded as an α-glucoside, both from its optical properties and also from the behaviour of sucrose towards the α-glucosidase, maltase, by which it is hydrolysed. Willstätter has shown that yeast maltase can be freed from invertase, and Weidenhagen [79] has demonstrated that at the optimum $p\mathrm{H} = 7$ maltase (free from invertase) is able to hydrolyse sucrose and maltose at about the same velocity, whereas at $p\mathrm{H} = 4{\cdot}6$, which is the optimum for invertase, there is no hydrolysis.

The nature of the fructose constituent presents greater difficulty. From its behaviour to enzymes it may be regarded provisionally as β-fructoside.

Invertase, according to Schlubach and Rauchalles [80] hydrolyses sucrose and β-methylfructofuranoside at much the same rate, and Weidenhagen [81] also regards invertase as β-fructosidase.

Sucrose has not yet been synthesised. Earlier claims to this end have all been discredited, and this also applies definitely to that of Pictet and Vogel [82] in 1928, which has to some extent unfortunately passed into the literature. If Haworth's formula is correct, a successful synthesis demands the union of α-glucopyranose with probably β-fructofuranose.

The coupling of glucopyranose with fructopyranose cannot give sucrose : this explains the many failures in the past by careful workers to effect the synthesis from such components.

Even the condensation of glucopyranose with fructofuranose is by no means a simple process since these components may give rise to a mixture of four " sucroses," i.e.,

1. α-glucose-β-fructose,
2. α-glucose-α-fructose,
3. β-glucose-α-fructose,
4. β-glucose-β-fructose,

or to three trehaloses αα, ββ, and αβ by condensation of two glucose components together, or to three isomeric difructoses by condensation of the fructose components in the same way.

It is not yet possible to obtain components or to devise conditions which will lead to the production of a unique product in these condensations : rather one is to expect a mixture of ten different substances.

Pictet and Vogel employed a crude mixture of fructose acetates to couple with glucose : it has been shown to contain very little of the desired fructofuranose constituent.[83] Irvine has sought to couple tetracetyl-γ-fructose with tetracetylglucose in presence of a dehydrating agent, both these compounds being syrups, presumably mixtures of α- and β-forms. Irvine has used three different sources for the fructose derivative, namely, syrups derived

(*a*) from inulin,
(*b*) from γ-ethylfructoside,
(*c*) from the hydrolysis of sucrose octacetate,

hoping thus to obtain the modification present in sucrose. He also condensed tetracetylchlorofructose with acetylglucose in the presence of a base.

Sucrose octacetate is split by acetylbromide dissolved in glacial acetic acid to an equimolecular mixture of the two acetates. All attempts to recondense these constituents by means of phosphoric anhydride failed to give sucrose octacetate, only the *iso*sucrose derivative with occasionally some *iso*trehalose octacetate. In every case the only compound isolated was *iso*sucrose-octacetate, m.p. 129°-131°, $[\alpha]_D$ + 19·3°, whereas sucrose octacetate has $[\alpha]_D$ + 59·6°.

*Iso*sucrose is less stable than sucrose, and it is therefore regarded as improbable that sucrose octacetate is formed first and subsequently changes into *iso*sucrose.

Irvine regards *iso*sucrose as β-glucose-β-γ-fructoside, that is to say, it is the glucose half of the molecule which has been wrongly coupled in the stereochemical sense. He has shown that fully methylated *iso*sucrose yields on hydrolysis tetramethyl glucopyranose and tetramethyl fructofuranose.

It is equally impossible to accept Pictet and Vogel's [84] claim to have synthesised the three other isomerides of sucrose.

## Trisaccharides and Tetrasaccharides.

Whereas in cellulose, starch and inulin a single hexose unit is repeated many times in the long chain of the polysaccharide molecule, there exist sugars in which several different hexose units are conbined to tri- and tetrasaccharides.

Only a limited number are known, but it is probable that others are still to be isolated, particularly from among the saponin glycosides. Little is known of the manner of formation of these compounds in plants or their function, and interest in them largely centres round the order in which the constituent units are arranged and the manner in which they are broken down by enzymes, as a particular linkage is broken only by the appropriate enzyme.

| | Oligosaccharides. | $[\alpha]_D$. |
|---|---|---|
| Reducing. | | |
| Manninotriose | Glucose-galactose-galactoside<br>6 ←→ 1 6 ←→ 1 | + 167° |
| Rhamninose . | Galactose-rhamnose-rhamnoside | − 41° |
| Robinose . | Galactose + 2 rhamnose | + 5° |
| Non-Reducing. | | |
| Raffinose . | 2-β-fructofuranose-1-α-glucose-6-α-galactoside | + 104° |
| Melezitose . | 1-α-glucose-2-β-fructofuranose-6-α-glucoside | + 88·7° |
| Gentianose . | 2-β-fructofuranose-1-α-glucose-6-β-glucoside | + 33° |
| Stachyose . | Fructofuranose-glucose-galactose-galactoside | + 144° |

A new interest is likely to attach to oligosaccharides following their discovery as constituents of the tubercle bacillus. Thus Mont and Anderson [85] have found trisaccharides, at present ill-characterised and of doubtful purity, containing mannose, *d*-arabinose and inositol; mannose and inositol; mannose, *d*-arabinose and glucose, some of which give positive precipitin reactions in serological tests and are therefore important for the study of immunity.

## Raffinose.

Galactose-glucose-fructose.

$\alpha I \leftarrow\rightarrow 6 \quad \alpha I \leftarrow\rightarrow 2\beta$

The best-known trisaccharide is raffinose, m.p. 118°-119°, $[\alpha]_D$ + 104° (hydrate), often found in considerable amount in the sugar beet, and present accompanying sucrose in other plants, particularly the seeds of cereals and in other seeds, leaves, branches and roots, and in exudations caused by injury by insects. The best source for the preparation of raffinose is cotton-seed meal, which contains it to the extent of nearly 8 per cent.: this proportion of the cotton-seed cake that is produced annually in the United States amounts to 100,000 tons. The raffinose is extracted from the meal with water, and after purification by means of its barium salt, may be isolated from the latter by exact neutralisation of the barium with phosphoric acid. The preparation, with a yield of 2·25 per cent. of the meal taken, is described by Harding.[86]

Raffinose has no reducing power. Strong mineral acids hydrolyse it completely to fructose, glucose and galactose in equal proportions; dilute acids form melibiose and fructose. The action of enzymes on raffinose is more specialised; invertase converts it into fructose and melibiose. Emulsin containing α-galactosidase, however, hydrolyses it to sucrose and galactose. Bottom yeasts which contain both α-galactosidase and invertase are able to ferment it completely.

The constitutional formula may be written

$$\underbrace{C_6H_{11}O_5\text{—O—}C_6H_{10}O_4}_{\text{Sucrose.}}\text{—O—}\underbrace{C_6H_{11}O_5}_{\text{Melibiose.}}$$

Fructose Glucose Galactose

It is α-melibiosido-fructose or α-galactosido-sucrose.

Hydrolysis of hendecamethyl raffinose gives 2 : 3 : 4-trimethyl glucose, 2 : 3 : 4 : 6-tetramethylgalactose and 1 : 3 : 4 : 6-tetramethyl-fructose, confirming the above formulation.[87]

## Verbascose.

Isomeric with raffinose is a sugar, verbascose, occurring in the roots of mullein, *Verbascum thapsus*.[88] It crystallises in aggregates of slender needles, m.p. 219°-220°, $[\alpha]_D$ + 169·9°, and yields glucose, fructose and galactose on hydrolysis. Invertase slowly splits off fructose.

## Gentianose.

Fructose-glucose-glucose.

$\beta 2 \longleftrightarrow 1\alpha \quad 6 \longleftrightarrow 1\beta$

Gentianose, m.p. 209°-211°, $[\alpha]_D$ + 33°, is obtained from the rhizomes of *Gentian* species.

The air-dried gentian root powder contains 24 per cent. of gentianose, of which 96 per cent. is extracted with 90 per cent. alcohol.[89] Only some 26 per cent. is extracted by stronger 95 per cent. alcohol. Gentianose is non-reducing, and is hydrolysed by invertase or very dilute acids to fructose and gentiobiose. Some emulsin preparations, and in particular extracts of *Aspergillus niger*, convert it into glucose and sucrose (Bourquelot). According to Bridel, the reversible breakdown of gentianose to glucose and sucrose in the roots of gentians is governed by the activity of the enzyme gentiobiase; there is every likelihood that this enzyme is merely β-glucosidase. Stronger acids hydrolyse it to a mixture of fructose and two molecules of glucose having $[\alpha]_D$ − 20·2°. Mammalian enzymes are without action, but those of molluscs and crustaceæ, particularly of the snail, act firstly to eliminate fructose and then hydrolyse the gentiobiose.

The constitutional formula is thus written

$$\underbrace{C_6H_{11}O_5\text{—}O\text{—}C_6H_{10}O_4}_{\text{Sucrose.}}\text{—}O\text{—}\underbrace{C_6H_{11}O_5}_{\text{Gentiobiose.}}$$

Fructose Glucose Glucose

Gentianose is thus α-gentiobiosido-fructose or β-glucosido-sucrose.

## Melezitose.

Glucose-fructose-glucose.

$\alpha 1 \longleftrightarrow 6 \quad \beta 2 \longleftrightarrow 1\alpha$

Melezitose (melicitose), m.p. 148°-150°, $[\alpha]_D$ + 88·5°, is found in the sweet exudations of the bark and leaves of many kinds of plant, such as in the "honey-dew" of limes and poplars, in Briançon manna,

which is exuded from the young twigs of the larch, and as a gummy secretion on bamboo stalks.

Hudson and Sherwood [90] have obtained it in quantity from the manna exuded by the Douglas fir tree, which contains as much as 75 per cent. of the trisaccharide. It is also present to the extent of 30 per cent. in Turkestan manna from *Alhagi camelorum*.

The melezitose containing exudations are produced as a result of the attacks of scale insects or of aphids. It is not known whether melezitose actually occurs in the normal cell sap or whether it is only formed as a result of injury. The honey-dew is collected by bees instead of flower nectar, with the result that honey often contains considerable amounts of melezitose,[91] which, unlike sucrose, resists hydrolysis by invertase.

Hudson and Sherwood [92] found as much as 20 per cent. melezitose in comb honey collected by bees from *Pinus virginiana*. In 1917 a great deal of melezitose honey was collected by bees in the eastern United States owing to the failure of blossoms in a dry summer. The melezitose crystallised in the comb, and many bees, deprived of their winter food reserves, died. It is doubtful whether bees have enzymes enabling them to hydrolyse and so utilise melezitose.[93]

Melezitose does not reduce Fehling's solution, or exhibit mutarotation, or form a phenylosazone. Dilute acids, e.g. 20 per cent. acetic acid, hydrolyse it to turanose and glucose, the rotation falling to $+ 63°$. Stronger acids give rise to fructose (one molecule) and glucose (two molecules). It forms a hendecacetate, m.p. 117°, $[\alpha]_D + 110°$.

While it is not hydrolysed by invertase or fermented by ordinary yeast, melezitose is easily hydrolysed by weak acids, the hydrolysis rate definitely placing it in a class with the $\gamma$-fructosides, cane sugar and raffinose.[94]

The reason for the discrepancy is clear: invertase is a fructosaccharase compatible with the fructose component of sucrose, but unable to act when the fructose is further substituted as in melezitose. The mixture of enzymes from *Aspergillus oryzæ* (Taka diastase) hydrolyse melezitose completely, it being impossible to say whether the sucrose or turanose link is first attacked. The enzymes here effective are gluco-saccharases or $\alpha$-glucosidases and can therefore act on melezitose. The evidence from the action of enzymes is that melezitose is a substituted sucrose.

Methylation, followed by hydrolysis, yields 2 : 3 : 4 : 6-tetramethylglucose (two molecules) and 1 : 3 : 4-trimethylfructose.[61]

This, together with Bridel's finding that turanose is an α-glucosido-fructose, enables melezitose to be written

$$\underset{\text{Glucose}}{\overset{1\leftarrow\rightarrow 6}{\text{Glucose}}}\underset{\text{Turanose.}}{\text{—Fructose}}\underset{\text{Sucrose.}}{\overset{2\leftarrow\rightarrow 1}{\text{—Glucose}}}$$

The alternative formula preferred by Pacsu takes no account of the sensitivity to acids shown by melezitose.

## Rhamninose.

Rhamninose, $C_{18}H_{34}O_{14}$, m.p. 135°-140°, $[\alpha]_D$ — 41°, is derived from the glycoside xanthorhamnin present in the Persian berry (*Rhamnus infectoria*).[95] The berries also contain an enzyme, rhamninase, which resolves the glycoside into the trisaccharide and rhamnetin. The carbohydrate forms colourless crystals which are somewhat sweet: it reduces Fehling's solution. On hydrolysis by mineral acids galactose and rhamnose (two molecules) are formed. The galactose is considered to be the terminal unit since the rhamninitol and rhamninonic acids, formed by reduction and oxidation respectively, are hydrolysed by acids to dulcitol or galactonic acid and rhamnose (two molecules). Rhamninose is not fermentable and the ordinary enzymes are without action. It appears to be slowly hydrolysed by the intestinal juice of *Helix*.

The formula may be written

$$\underset{\text{Galactose.}}{C_6H_{11}O_5}\text{—O—}\underset{\text{Rhamnose.}}{C_6H_{10}O_3}\text{—O—}\underset{\text{Rhamnose.}}{C_6H_{11}O_4}$$

## Robinose.

This sugar is described as isomeric with rhamninose by Charaux.[96] It is obtained from the glycoside robinin, together with kæmpferol, on hydrolysis with rhamninase. The sugar is hygroscopic and exhibits mutarotation to a final value of $[\alpha]_D$ + 1·9°. It is hydrolysed to galactose (one molecule) and rhamnose (two molecules).

## Manninotriose.

Manninotriose, m.p. 150°, $[\alpha]_D$ + 167°, tasting faintly sweet, has been found only in ash manna, the dried exudation from the bark of *Fraxinus ornus*,[97] in which it is accompanied by manninotetrose or stachyose, and from which it may be obtained by the action of invertase or of dilute acetic acid.

It reduces Fehling's solution and forms a phenylosazone, m.p. 122°-124°.

Manninotriose is hydrolysed by acids to glucose (one molecule) and galactose (two molecules). Bromine oxidises it to manninotrionic acid, which is hydrolysed by acids to gluconic acid and galactose, thus locating the glucose molecule at the end of the chain. The action of enzymes on manninotriose is still a matter of uncertainty. Bierry has shown that the intestinal juice of the snail probably first forms galactose and a disaccharide, glucose + galactose, which is subsequently hydrolysed. According to Neuberg and Lachmann glucose and a digalactose are formed by the action of almond emulsin.

$$\underset{\text{Glucose}}{CHO\,.\,C_5H_{10}O_4}—O—\underset{\text{Galactose}}{C_6H_{10}O_4}—O—\underset{\text{Galactose}}{C_6H_{11}O_5}$$

Gluco-galactose. Digalactose.

## Stachyose.

The tetrasaccharide stachyose (manninotetrose, lupeose), $C_{24}H_{42}O_{21}$, is found in ash manna, in the twigs of white jasmine, in the seeds of many *Leguminosæ*, in the roots and rhizomes of *Labiates*, specially *Stachys tuberifera*. It forms an insoluble strontium salt which enabled Tanret [98] to isolate it from *Leguminosæ*.

Stachyose tastes quite sweet, has m.p. 167°-170° and $[\alpha]_D$ + 148°. It has no reducing properties. Onuki [99] describes its preparation from *Stachys tuberifera*.

Strong acids hydrolyse stachyose to two molecules of galactose, and one of glucose and fructose ; acetic acid or invertase yield fructose and manninotriose.

As a result of the work of Onuki,[100] stachyose may be regarded as a galactosido-raffinose, an additional molecule of galactose being attached to the galactose already present in raffinose.

Stachyose forms a tetratrityl derivative, and therefore has four $CH_2OH$ groups. Methylation of stachyose and manninotriose gave the partially methylated galactose, glucose and fructose corresponding to the following formula :—

Raffinose.

Galactose—Galactose—Glucose—Fructose (1 : 4)
1 ⟷ 6 1 ⟷ 6 1 ⟷ 2

Sucrose.

The α or β nature of the linkages is yet to be fully established.

## REFERENCES TO CHAPTER XV.


1. HAWORTH AND PEAT, J.C.S., 1926, 3094.
2. ZEMPLEN, Ber., 1926, **59**, 1254; Ber., 1927, **60**, 1555.
3. BRIDEL, Bull. Soc. Chim. biol., 1925, **7**, 181.
4. GILLOT, J. Pharm. Chim. (7), 1923, **28**, 148.
5. COLIN AND FRANQUET, Compt. rend., 1928, **186**, 890.
6. MEUNIER, Compt. rend., 1933, **197**, 98.
7. IRVINE AND BLACK, J.C.S., 1926, 862. COOPER, HAWORTH AND PEAT, J.C.S., 1926, 876, 3094.
8. FREUDENBERG, W. KUHN, DÜRR, BOLZ AND STEINBRUNN, Ber., 1930, **63**, 1510.
9. FREUDENBERG, FRIEDERICH AND BUMANN, Ann., 1932, **494**, 41.
10. FISCHER, Ber., 1890, **23**, 3687; Ber., 1895, **28**, 3024.
11. SCHEIBLER AND MITTELMEIER, Ber., 1891, **24**, 301.
12. HARRISON, J.A.C.S., 1914, **36**, 586.
13. CROFT HILL, J.C.S., 1903, 578.
14. ARMSTRONG, Proc. Roy. Soc., 1905, **76 B**, 592.
15. HAWORTH, J. Soc. Chem. Ind., 1927, **46**, 295.
16. BERLIN, J.A.C.S., 1926, 1107; 2627.
17. GEORG AND PICTET, Helv. Chim. Acta, 1926, **9**, 612.


### CELLOBIOSE.


18. FRANCHIMONT, Ber., 1879, **12**, 1941.
19. SKRAUP, Monatsh., 1901, **22**, 1011.
20. HAWORTH AND HIRST, J.C.S., 1921, 193.
21. HAWORTH, LONG AND PLANT, J.C.S., 1927, 2809.
22. KARRER AND TSCHAN, Helv. Chim. Acta, 1926, **9**, 680.
23. BERGMANN AND BREUERS, Ann., 1929, **470**, 38.
24. HUDSON, J.A.C.S., 1926, 2002.
25. FREUDENBERG AND NAGAI, Ber., 1933, **66**, 27.
26. FREUDENBERG, ANDERSON AND GO, Ber., 1930, **63**, 1961.
27. FREUDENBERG AND KUHN, Ber., 1932, **65**, 484.
28. BERTRAND AND BENOIST, Compt. rend., 1923, **176**, 1583. IRVINE, J. Soc. Chem Ind., 1925, 44, 242. OST, Z. angen. Chem., 1926, **39**, 117.
29. WILLSTÄTTER AND ZECHMEISTER, Ber., 1929, **62**, 722. ZECHMEISTER AND TOTH, Ber., 1931, **64**, 857. ZECHMEISTER, MARK AND TOTH, Ber., 1933, **66**, 269.
30. FREUDENBERG AND FRIEDRICH, Ber., 1930, **63**, 1963.
31. HAWORTH, HIRST AND ANT-WUORINEN, J.C.S., 1932, 2368.
32. FREUDENBERG AND NAGAI, Ann., 1932, **494**, 63.
33. BERGMANN AND MACHEMER, Ber., 1926, **59**, 991.
34. HAWORTH AND MACHEMER, J.C.S., 1932, 2372.


### LACTOSE


35. HAWORTH AND LONG, J.C.S., 1927, 544.
36. RUFF AND OLLENDORF, Ber., 1900, **33**, 1802.
37. ZEMPLEN, Ber., 1926, **59**, 2402.
38. KING AND HUDSON, J.A.C.S., 1926, 1978; 2435.
39. MONTGOMERY AND HUDSON, J.A.C.S., 1930, 2101.
40. FISCHER AND ARMSTRONG, Ber., 1902, **35**, 3144.
41. POLONOVSKI AND LESPAGNOL, Compt. rend., 1931, **192**, 1319; Compt. rend. 1932, **195**, 465.
42. HELFERICH AND SPARMBERG, Ber., 1933, **66**, 806.

GENTIOBIOSE AND PRIMEVEROSE.

43. Bergmann and W. Freudenberg, Ber., 1929, **62**, 2783.
44. Haworth and Wylam, J.C.S., 1923, 3120.
45. Bourquelot and Hérissey, Compt. rend., 1913, **157**, 732.
46. Helferich, Klein and Schäfer, Ann., 1926, **447**, 19.
47. Haworth, Loach and Long, J.C.S., 1927, 3146.
48. Goris, Mascre and Vischniac, Bull. Sci. Pharm., 1912, **19**, 577; 1920, **27**, 258.
49. Helferich and Rauch, Ann., 1927, **455**, 168.

MELIBIOSE AND VICIANOSE.

50. Harding, Sugar, 1923, **25**, 514.
51. Helferich and Bredereck, Ann., 1928, **465**, 166.
52. Helferich and Rauch, Ber., 1926, **59**, 2655.
53. Pictet and Vogel, Helv. Chim. Acta, 1926, **9**, 805; Helv. Chim. Acta, 1927, **10**, 280.
54. Challinor, Haworth and Hirst, J.C.S., 1931, 258.
55. Bertrand and Weisweiler, Bull. Soc. Chim., 1911, **9**, 38; 84; 147.

TURANOSE.

56. Alekhine, Ann. Phys. Chim., 1889, **18**, 532.
57. Tanret, Bull. Soc. Chim., 1906, **35**, 816.
58. Hudson and Pacsu, J.A.C.S., 1930, **52**, 2519.
59. Bridel and Aagaard, Compt. rend., 1927, **184**, 1667; Bull. Soc. Chim. biol., 1927, **9**, 884.
60. Aagaard, Tidsskr. Kjemi. Berg., 1928, **8**, 5; 16; 35.
61. Zemplen and Braun, Ber., 1926, **59**, 2230; 2539. Leitch, J.C.S., 1927, 588.
62. Pacsu, J.A.C.S., 1931, 3099.
63. Jacobs and Hoffmann, J.B.C., 1926, **67**, 609; **69**, 153; 1928, **79**, 531.
64. Charaux, Compt. rend., 1924, **178**, 1312; 1925, **180**, 1419.

TREHALOSE.

65. Harding, Sugar, 1923, **25**, 476.
66. Bredereck, Ber., 1930, **63**, 959.
67. Fischer and Delbrück, Ber., 1909, **42**, 2783.
68. Haworth and Hickinbottom, J.C.S., 1931, 2847.
69. Vogel and Kurnicka, Helv. Chim. Acta, 1928, **11**, 910.
70. Robison and Morgan, Biochem. J., 1928, **22**, 1277.
71. Power and Salway, J.C.S., 1914, 767; 1062.
72. Buston and Schryver, Biochem. J., 1923, **17**, 470.

SUCROSE.

73. Cox and Metschl, Ind. Eng. Chem. News. Edition, 1932, p. 149.
74. Thomas and Schuette, J.A.C.S., 1931, 2324.
75. Berner, Ber., 1933, **66**, 1076.
76. Haworth and Law, J.C.S., 1916, 1314.
77. Irvine and Stiller, J.A.C.S., 1932, 1486.
78. Haworth, Ber., 1932, **65**, 50.
79. Weidenhagen, Naturwiss., 1928, **16**, 654.
80. Schlubach and Rauchalles, Ber., 1925, **58**, 1842.
81. Weidenhagen, Z. Ver. deut. Zuckerind., **80**, 376.
82. Pictet and Vogel, Helv. Chim. Acta, 1928, **11**, 436; Ber., 1929, **62**, 1418.

83. IRVINE, OLDHAM AND SKINNER, J.A.C.S., 1929, 1279. IRVINE AND OLDHAM, J.A.C.S., 1929, 3609. ZEMPLEN AND GERECS, Ber., 1929, **62**, 984.
84. PICTET AND VOGEL, Helv. Chim. Acta, 1928, **11**, 901.

OLIGOSACCHARIDES.

85. MONT AND ANDERSON, Z. Physiol. Chem., 1932, **211**, 97; 103.
86. HARDING, Sugar, 1923, **25**, 308.
87. HAWORTH, CHARLTON AND HICKINBOTTOM, J.C.S., 1927, 1527.
88. BOUQUELOT AND BRIDEL, Compt. rend., 1910, **151**, 760.
89. BRIDEL AND DESMAREST, J. Pharm. Chim., 1929, **9**, 465.
90. HUDSON AND SHERWOOD, J.A.C.S., 1918, 1456. HARDING, Sugar, 1923, **25**, 240.
91. LUCIUS AND NOTTBOHM, Z. Unters. Lebensm., 1931, **61**, 165.
92. HUDSON AND SHERWOOD, J.A.C.S., 1920, 116.
93. PHILLIPS, Z. Unters. Lebensm., 1932, **64**, 383.
94. KUHN AND GRUNDHERR, Ber., 1926, **59**, 1655.
95. TANRET, Bull. Soc. Chim., 1899, **21**, 1065.
96. CHARAUX, Bull. Soc. Chim. biol., 1926, **8**, 915.
97. TANRET, Bull. Soc. Chim., 1902, **27**, 947.
98. TANRET, Compt. rend., 1912, **155**, 1562.
99. ONUKI, J. Agric. Chem. Soc. Japan, 1932, **8**, 445.
100. ONUKI, J. Agric. Chem. Soc. Japan, 1933, **9**, 90; 214.

## CHAPTER XVI.

### HYDROLYSIS AND SYNTHESIS OF OLIGOSACCHARIDES.

THE hydrolysis of di- and tri-saccharides into their component monosaccharides is effected by acids and also by the appropriate enzymes. With acids the rate of hydrolysis varies with the structure of the disaccharide, and with the strength of the acid. The hydrolytic action of enzymes is essentially selective, each particular type of glycosidic link being hydrolysed by the appropriate enzyme.

The hydrolysis of sucrose by acids has been a subject of great historical interest. The rapid hydrolysis, as compared with that of other sugars, for long appeared to demand an abnormal structure for sucrose. Now it is known that other sugars, raffinose and melezitose, which also, like sucrose, contain fructofuranose, are hydrolysed at much the same rate.

The hydrolysis rate of di- and tri-saccharides has been of some value for determining their structure, but as Moelwyn-Hughes [1] has pointed out, it is important that the conditions should be comparable.

Unless two compounds which are being compared have the same temperature coefficient for the hydrolysis rates, erroneous conclusions may be reached. For this reason the critical increment calculated according to the ordinary Arrhenius equation is a more significant constant for comparison, being independent of temperature.

The relative values of the unimolecular velocity constant at 60° C. and the critical increment for a number of disaccharides and trisaccharides are given :—[2]

| | K. | E. |
|---|---|---|
| Trehalose . | 59 | 40,180 |
| Lactose . | 1,138 | 27,100 |
| Maltose . | 1,150 | 31,500 |
| Melezitose | 338,300 | 25,600 |
| Raffinose . | 767,000 | 25,600 |
| Sucrose . | 1,000,000 | 26,000 |

Trehalose is the most resistant to hydrolysis, sucrose the least.

Freudenberg [3] has measured the rates of hydrolysis of cellobiose, turanose and melibiose.

Haworth [4] compares the relative velocity constant values for the hydrolysis of sucrose and furanosides with certain pyranosides, proving that sucrose is a typical furanose in this characteristic:—

| Pyranosides. | | Furanosides. | |
|---|---|---|---|
| α-Methylglucoside | 100 | α-Methylglucoside | 13,400 |
| β-Methylglucoside | 120 | α-Ethylglucoside | 40,000 |
| α-Methylmannoside | 40 | β-Ethylglucoside | 20,960 |
| | | α-Methylmannoside | 6,000 |
| | | Sucrose | 20,000 |

## The Hydrolysis by Enzymes.

The hydrolysis of oligosaccharides by enzymes is specific in so far as a particular enzyme is specific for a particular type of glycosidic link, but an enzyme may hydrolyse a series of sugars which contain this type of link.

Sucrose is both a glucoside and a fructoside and is hydrolysed by two different enzymes. Similarly, the trisaccharides raffinose and gentianose, are hydrolysed by invertase in virtue of the sucrose residue contained in them, but also by α-galactosidase and β-glucosidase respectively.

The following sugars are hydrolysed by invertase:—

Sucrose = fructose < > glucose

Gentianose = fructose ↗ < > glucose > glucose

Raffinose = fructose ↗ < > glucose > galactose

Stachyose = fructose ↗ ↗ < > glucose > galactose > galactose

The following by the β-glucosidase present in emulsin:—

Gentianose = glucose < glucose < > fructose

Gentiobiose = glucose ↗ < glucose

Primeverose = xylose ↗ < glucose

Allolactose = galactose ↗ < glucose

Cellobiose = glucose ↗ < glucose

* Lactose = galactose ↗ < glucose

The following by maltase (α-glucosidase):—

Sucrose = glucose < > fructose

Melezitose = glucose ↗ < > fructose > glucose ↖

Turanose = ↗ fructose > glucose ↖

* According to Helferich lactase = β-galactosidase = β-glucosidase.

The following by α-galactosidase (melibiase), contained in crude emulsin and bottom yeasts :—

Raffinose = galactose < glucose < > fructose
Melibiose = galactose < glucose
Vicianose = *l*-arabinose < glucose.

Trehalose as α-glucosido-α-glucoside should be hydrolysed by maltase, but maltase preparations are known which split maltose but not trehalose, and trehalase preparations from fungi which split trehalose but not maltose. Trehalase therefore seems a definite enzyme. The action of emulsin on trehalose is attributed to the α-glucosidase it contains.

The action of enzymes on trehalose as well as on the synthetic *iso*trehaloses is worthy of reinvestigation.

## The Synthesis of Disaccharides.

Although in the hands of Fischer the problem of the synthetical preparation of the simple monosaccharides was solved, the next step, the synthesis of disaccharides, has proved more difficult.

The earliest synthetical disaccharide was obtained by Fischer [5] by the action of cold concentrated hydrochloric acid on glucose. The syrupy compound obtained was termed *iso*maltose on account of certain resemblances to maltose, from which it differed in being non-fermentable. The product was undoubtedly a complicated mixture : it certainly contains some gentiobiose, and perhaps also some maltose. A more practical method, based on Michael's glucoside synthesis, appeared to be the combination of acetochloroglucose with the sodium salt of a hexose. In this way Fischer and Armstrong [6] obtained disaccharides of the reducing type which they termed galactosido-glucose and glucosido-galactose. These sugars were sufficiently similar to natural disaccharides to be hydrolysed by enzymes. Top yeast was without action, bottom yeast was able to ferment both disaccharides : they were hydrolysed by emulsin, but not affected by maltase or invertase. Both reduced Fehling's solution and formed phenylosazones, but they could not be obtained crystalline. The galactosido-glucose was thought to possess a similarity to melibiose. Schlubach and Rauchenberger [7] later have considered it to be impure lactose.

Condensations are more successful with sugar derivatives in which the number of free hydroxyl groups is limited. Thus Irvine [8] ob-

tained an isomer of trehalose by the action of 0·25 per cent. hydrogen chloride on tetramethylglucose in benzene.

The synthesis of sucrose by the action of phosphorus pentoxide on a mixture of glucose and fructofuranose tetracetates has been claimed by Pictet,[9] but in fact only produces *iso*sucrose derivatives. A chemical synthesis of sucrose remains to be achieved.

Pictet [10] claims to have synthesised several disaccharides by thermal condensation of the sugars or of their anhydrides, for example, maltose from an equimolecular mixture of α- and β-glucosan, melibiose,[11] lactose [12] from diglucosan and β-galactose. The method was improved by heating the sugars or the glucosans with zinc chloride *in vacuo*. Thus β-glucose with α-glucose or α-glucosan gives maltose; [13] β-glucose with β-galactose or β-galactosan gives lactose.[14]

A most useful synthetic method, which at the same time proves the structure of the synthesised disaccharide, is due to Helferich.[15]

The first step is to form the trityl derivative of a sugar protecting the $-CH_2OH$ group by reacting with triphenylmethyl chloride: after acetylation the trityl group is removed with acids leaving the $-CH_2OH$ alone free. An acetohalogeno sugar, either a hexose or a pentose, is then condensed with the 1 : 2 : 3 : 4-tetracetyl sugar to give the disaccharide. The ordinary conditions for glycoside condensation give a β-glycosidic disaccharide linkage, but they can be altered to give the α-glycoside.

In this way have been synthesised gentiobiose and primeverose, vicianose, melibiose and 6-β-galactosido-glucose.

Freudenberg [16] has used diacetone galactose, in which the $-CH_2OH$ group is free, to condense with various acetohalogeno sugars, in this way synthesising crystalline 6-glucosido-galactose, 6-galactosido-galactose and 6-mannosido-galactose, and 6-cellobiosido- and 6-lactosido-galactose as syrups.

Acetobromoglucose adds to lævoglucosan to give an intermediate product which in 50 per cent. sulphuric acid yields the tetracetate, and after acetylation the octacetate of cellobiose.[17]

The synthesis of octamethyl cellobiose is achieved by the condensation of 2 : 3 : 6-trimethylglucose-β-methylglucoside with 2 : 3 : 4 : 6-tetramethylglucose 1-chlorhydrin.[18]

New disaccharides may be obtained by conversion of an already known disaccharide into its epimer, either by the Lobry de Bruyn conversion, or by converting first into the glycal and oxidation with perbenzoic acid. Thus the glucose residues in cellobiose and lactose can be converted into mannose residues via cellobial and lactal.[19]

The following disaccharides which have no natural representation have been synthesised :—

| Name. | $[\alpha]_D$. |
|---|---|
| Glucose-6-α-glucoside [20] | + 11° |
| Glucose-6-β-galactoside [21] | + 36° |
| Galactose-6-β-galactoside [22] | + 34° |
| Galactose-6-glucoside [23] | + 8·2° |
| Mannose-4-glucoside [19] | + 10·7° |
| Mannose-4-galactoside [19] | + 30° |
| Galactose-6-Mannoside [22] | + 134° |
| Mannose-mannoside [24] | + 13° |
| Arabinose-*d*-galactoside [25] | — 63° |

TRISACCHARIDES.

| Name. | $[\alpha]_D$. |
|---|---|
| Maltose-β-glucoside [26] | + 160° |
| Glucose-6-β-lactoside [27] | + 23° |
| Galactose-6-lactoside [22] | + 22° |
| Glucose-6-β-cellobioside [22] | + 8° |
| Galactose-6-cellobioside [22] | + 25° |

## Synthesis by Enzymes.

Important as chemical synthesis is for the determination of the structure of the disaccharides and trisaccharides, the syntheses which have been achieved by means of enzymes are of much greater interest to the biologist, since there is no doubt that in the plant, enzymes function as synthetical agents.

The first to observe the synthetical or, as he termed it, reversible action of enzymes was Croft Hill.[28] Hill proved that the hydrolysis of maltose by dried yeast extract in concentrated solutions was not complete and that, starting from glucose alone in concentrated solution, a disaccharide was produced by the action of maltase. This sugar he at first considered to be maltose, a conclusion disputed by Emmerling,[29] who, repeating Croft Hill's experiments, considered the product to be *iso*maltose identical with that obtained by Fischer by the action of acid on glucose. Subsequently Croft Hill [30] admitted the chief product to be an isomeride of maltose, but he regarded it as different from Fischer's *iso*maltose and termed it revertose. He still claimed that maltose was also formed in small quantity. E. F. Armstrong [31] considered that the product of the synthetical action of maltase on glucose was *iso*maltose identical with that produced by the action of hydrochloric acid on glucose.

Pringsheim [32] was actually able to isolate crystalline maltose, together with other uncrystallisable sugars, from the action of yeast maltase on glucose, and found that a β-glucosidase contained in bottom yeast was able to cause the synthesis of gentiobiose.

Proof was given by Bourquelot [33] and confirmed by Zemplen that gentiobiose is the product of the condensation of glucose in presence of emulsin. Both have isolated the sugar in a crystalline state, and to Bourquelot belongs the credit of the first synthesis of a crystalline natural disaccharide.

Bourquelot and Aubry [34] have obtained two crystalline mutarotating galactobioses by the action of emulsin on concentrated galactose solutions: Bourquelot and Hérissey [35] obtained a mannobiose from mannose in the same way.

The process by which a monosaccharide is converted into a disaccharide in presence of a synthetical catalyst must be regarded as precisely similar to that by which α- and β-glucoses are converted into the two methylglucosides.

The process is complicated by the fact that the first molecule offers a number of hydroxyl groups as alternatives for the aldehyde group of the second molecule to condense with: consequently a mixture should and does result, as in the action of acids on glucose, when gentiobiose, maltose and *iso*maltose are among the products. The isomers produced may differ not only in the carbon atom at which the disaccharide link is formed but in the α or β configuration of the link. The rates of formation of the isomers will be different, in favourable cases so different as to cause the virtual exclusion of all but one compound, which can then be isolated pure.

The use of an enzyme catalyst will determine the α- or β-linkage, according to its nature, but not necessarily the carbon atom at which the linkage is formed. With emulsin, however, it is gentiobiose, i.e. glucose-6-β-glucoside, which is synthesised, rather than cellobiose, i.e. glucose-4-β-glucoside, or other isomers, which may be formed in traces. A more complete analysis of a disaccharide mixture resulting from an enzyme synthesis, is much wanted in order to establish this question of enzyme selectivity in synthesis.

With invertase the evidence is that this enzyme from yeast effects a complete hydrolysis to glucose and fructose of the sucrose and there is no detectable reverse reaction. The reason is now clear in that the fructofuranose first produced on hydrolysis reverts to fructopyranose, and consequently the amount of fructofuranose available for the reverse reaction is for practical purposes nil.

The hydrolysis of sucrose was investigated with great care by H. E. Armstrong in 1901, and by Hudson in 1914 with all the refinement which modern methods of experiment permitted.

Conditions are stated to be somewhat different when the hydrolysis

is effected in the presence of a phosphate such as $Ca(H_2PO_4)_2$. The calcium sucrose phosphate is hydrolysed by invertase in the first place to glucose and calcium-$\gamma$-fructose phosphate. Under the influence of phosphatase there is an equilibrium between

Ca-$\gamma$-fructose phosphate $\rightleftarrows$ calcium phosphate + $\gamma$-fructose.

The hydrolysis is never complete, and starting from glucose and fructose in presence of invertase and phosphatase there is evidence of 20 per cent. synthesis according to Oparin and Kurssanow.[36]

These investigators were unable to isolate the synthetic sucrose, but it behaved optically as if it were sucrose. A confirmation of these important results is desirable.

The production of trehalose monophosphate by the action of dried yeast preparations may also be looked upon as an enzymic disaccharide synthesis.

It appears that raffinose can be synthesised by the action of emulsin on a mixture of sucrose and galactose. In aqueous solution the hydrolysis does not go to completion, and Blagoveschenski [37] found that in 80 per cent. acetone solution a synthesis occurred and was able to obtain a small quantity of crystals of $[\alpha]_D + 95{\cdot}7°$ (raffinose has + 104°, galactose + 81° and sucrose + 66·5°) which were hydrolysed by emulsin again. The compound has therefore the properties of raffinose.

## REFERENCES TO CHAPTER XVI.


1. Moelwyn-Hughes, Trans. Faraday Soc., 1928, **24**, 318.
2. Moelwyn-Hughes, Trans. Faraday Soc., 1929, **25**, 81.
3. Freudenberg, Dürr and Hochstetter, Ber., 1928, **61**, 1735.
4. Haworth, Ber., 1932, **61**, 50; J.C.S., 1932, 2254.
5. Fischer, Ber., 1890, **23**, 3687; Ber., 1895, **28**, 3024.
6. Fischer and Armstrong, Ber., 1902, **35**, 3144.
7. Schlubach and Rauchenberger, Ber., 1925, **58**, 1184; Ber., 1926, **59**, 2102.
8. Irvine, Biochem. Z., 1909, **22**, 367.
9. Pictet, Helv. Chim. Acta, 1928, **11**, 436.
10. Pictet, Bull. Soc. Chim., 1920, **27**, 650.
11. Pictet and Vogel, Helv. Chim. Acta, 1926, **9**, 806; Helv. Chim. Acta, 1927, **10**, 280.
12. Pictet, Arch. Sci. Phys. Nat., 1926, **43**, 179.
13. Pictet and Vogel, Helv. Chim. Acta, 1927, **10**, 588.
14. Pictet and Vogel, Helv. Chim. Acta, 1928, **11**, 209.
15. Helferich, Ann., 1926, **447**, 19; Ann., 1928, **465**, 166.
16. Freudenberg, Noë and Knopf, Ber., 1927, **60**, 239.
17. Freudenberg and Nagai, Ber., 1933, **66**, 27.
18. Freudenberg, Anderson and Go, Ber., 1930, **63**, 1961.
19. Bergmann and Schotte, Ber., 1921, **54**, 1564; Bergmann, Ann., 1923, **434**, 83.

20. PICTET AND CASTAN, Helv. Chim. Acta, 1921, **4**, 319.
21. HELFERICH AND RAUCH, Ber., 1926, **59**, 2625.
22. FREUDENBERG, Ber., 1928, **61**, 1743.
23. FREUDENBERG, Ber., 1927, **60**, 238.
24. PRINGSHEIM AND GENIN, Z. physiol. Chem., 1928, **140**, 299.
25. ZEMPLEN, Ber., 1927, **60**, 1309.
26. LING AND NANJI, J.C.S., 1923, 1666; 1925, 629.
27. HELFERICH AND SCHÄFER, Ann., 1926, **450**, 233.

## SYNTHESES BY ENZYMES.

28. CROFT HILL, J.C.S., 1898, 634.
29. EMMERLING, Ber., 1901, **34**, 600; 2206, 3810.
30. CROFT HILL, J.C.S., 1903, 578.
31. ARMSTRONG, Proc. Roy. Soc., 1905, **76 B**, 592.
32. PRINGSHEIM AND LEIBOWITZ, Ber., 1924, **57**, 1576. PRINGSHEIM, BONDI AND LEIBOWITZ, Ber., 1926, **59**, 1983.
33. BOURQUELOT, Compt. rend., 1913, **157**, 732.
34. BOURQUELOT AND AUBRY, Compt. rend., 1916, **163**, 60; Compt. rend., 1917, **164**, 443; 521.
35. BOURQUELOT AND HÉRISSEY, J. Pharm. Chim., 1920 (7), **21**, 81.
36. OPARIN AND KURSSANOW, Biochem. Z., 1931, **239**, 1.
37. BLAGOVESCHENSKI, Biochem. J., 1930, **24**, 1337.

# CHAPTER XVII.

## THE POLYSACCHARIDES.

In the preceding chapters frequent mention has been made of the polysaccharides as sources of the monosaccharides. While the scope of this book is restricted to the simpler carbohydrates, an account of them would not be complete without some description of their polymeric derivatives and of their structure, which has been deduced from the characterisation of the monosaccharide fragments obtained by hydrolysis, as well as by a variety of other methods, chemical, physical and enzymatic.

A brief account of the polysaccharides will therefore be given, and the currently accepted structures described, without going into the historical phases of the development of the subject, and without attempting to assess the contributions of individual workers in the field.

Polysaccharide chemistry presents many difficulties both of investigation and of classification.

Polysaccharides are substances of high molecular weight, forming colloidal solutions, and are difficult to obtain pure, and it is difficult to apply criteria for purity to them. An understanding of their physical properties only became possible as a result of the understanding of the nature of high polymers due in particular to the work of Staudinger,[1] and its application to naturally occurring polymeric substances, particularly by Meyer and Mark.[2]

The last decade has seen the rejection of the idea that high polymers consisted of aggregations of a large number of simple units held together only by secondary valency association forces, and its replacement by the view that they consist of simpler units joined by ordinary primary valencies, the same type of linkage between the units as between the atoms in the simple unit itself.

Most polysaccharides consist of long regular chains of monosaccharide units; other less well-investigated polysaccharides seem to contain, attached to each unit in the continuous chain, a second monosaccharide unit forming a branched chain. Whether polysaccharides

having long branched chains, that is to say, two dimensional as opposed to linear polymers, exist, is a question which only further experimental work can decide.

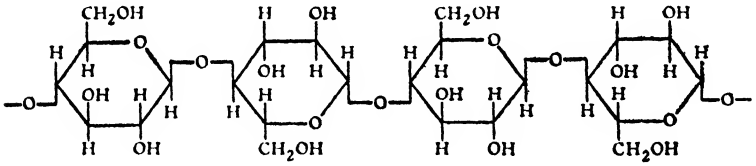
Cellulose comprises about one-half of the skeletal substances of green plants, in which it is accompanied by lignin and by a variety of hemicelluloses.

Chemical work has been carried out mainly with cotton cellulose, from the boll of the cotton plant, a somewhat specialised product. The use of the name cellulose is nowadays restricted by chemists to homogeneous preparations which yield glucose only on hydrolysis. The term hemicellulose is less well-defined ; it is used to include a number of polysaccharides probably of smaller molecular dimensions than cellulose, and which are on the whole more easily hydrolysed than cellulose. On hydrolysis of the hemicelluloses a variety of sugars are obtained, glucose, mannose, galactose, arabinose, xylose and glucuronic acid. Rarely is it possible to obtain preparations yielding but one sugar on hydrolysis such as xylan, which yields only xylose. More frequently mixtures of two or more sugars are obtained, and it is convenient to classify these hemicelluloses as proposed by Karrer,[3] according to the simple sugars they contain as hexosans, pentosans or mixed pentosan-hexosans, as gluco-mannosan or arabino-galactosan.

It is not yet established for most examples whether such compounds consist of mixtures of homogeneously constituted polysaccharides which it has not been possible to separate, or of heterogeneously constituted polysaccharides in which several different monosaccharide components linked together occur. Both types in fact exist.

## Cellulose.

Cellulose consists of a regular chain of $\beta$-glucose units joined in glucosidic union at carbon 4.



The evidence on which this formulation rests may be briefly summarised :—

Glucose is the only product of acid hydrolysis. Cellulose can be transformed successively into triacetylcellulose and into trimethylcellulose, and the latter yields on hydrolysis 2 : 3 : 6-trimethylglucose, every step giving a practically quantitative yield.

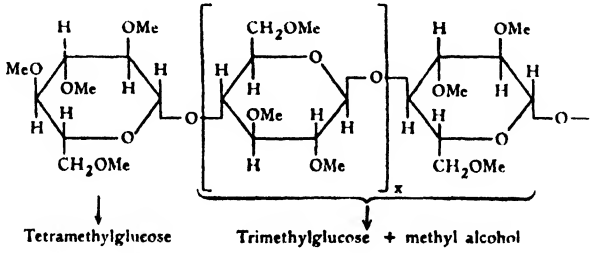
Mild conditions of hydrolysis of cellulose or its derivatives result in its breakdown through cellodextrins to the oligosaccharides, cellohexaose, cellotetraose, cellotriose and cellobiose and finally to glucose. The structure of cellobiose and cellotriose has been established by methylation, by degradation and by synthesis, while the structure of cellotetraose and cellohexaose as of identical type follows by inference from their properties. The methylated oligosaccharides are obtainable from trimethylcellulose, so the establishment of the pyranose ring structure for the former also establishes it for the latter.

A study of the kinetics of hydrolysis of cellulose fully confirms the above formulation, and rejects the idea that any other kind of glucosidic link or ring structure is present. The actual yields of cellobiose on acetolysis are in good agreement with those calculated.

X-ray studies of cellulose fibres confirm the result of chemical studies, and disclose a repeating unit of length 10·2 Å. which corresponds exactly to a cellobiose unit.

Haworth and Hirst [4] have determined the molecular dimensions of their sample of cellulose by an ingenious method.

It is evident that if fully methylated cellulose is hydrolysed the terminal unit should give rise to 2 : 3 : 4 : 6-tetramethylglucose, and it was actually found possible to identify and quantitatively separate the tetramethylglucose from the trimethylglucose in the hydrolysis products by distillation.




The amount of tetramethylglucose found corresponds to a chain length of from 100 to 200 glucose units, corresponding to a molecular weight of 16,000 to 32,000 and a molecule of length 500 to 1000 Å. or 5 to 10 $\times$ $10^{-6}$ cm.

As the methylated cellulose is obtained from cellulose via the

acetate, the chemical treatment may have caused some breaking down of the large molecule, so the method fixes a minimum molecular size.

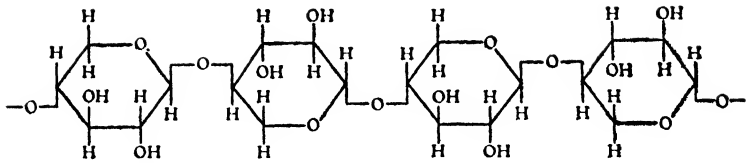
The molecular size of the cellulose molecule can also be estimated by a number of physical methods; osmotic measurements, diffusion experiments, X-ray methods and centrifugal methods all concur in giving a size corresponding to about 200 glucose units in the chain; only the Staudinger limiting viscosity method gives a greater size of 750 units, but there appear to be theoretical objections to this method.[5]

The nature of the terminal group at the potentially aldehydic end of the cellulose chain is undetermined. In the cellodextrins, produced on the degradation of cellulose, there is a free aldehyde group which can be titrated with hypoiodite and so used to give a measure of the molecular weight of the cellodextrin, which yields a figure in excellent agreement with that found by methylation. The method applied to cellulose is not considered reliable, and it gives the chain length as 50 units.

Schmidt [6] has used the carboxyl content of celluloses of various origins to estimate their chain length and so finds it to be about 100 units. The carboxyl groups may arise from oxidation of the terminal aldehyde group, but also from any one of the primary alcohol groups, so the method is subject to criticism.

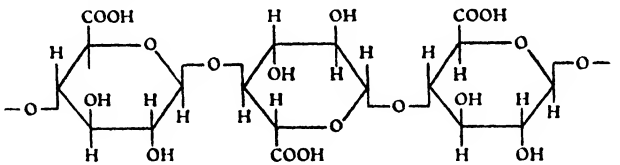
It will be of interest to determine by one method or another the dimensions of the celluloses from different sources. Other celluloses, particularly those from wood, are probably less truly homogeneous than cotton cellulose, and individual glucose units in the chain may have undergone degradation, by oxidation to glucuronic acid and decarboxylation to xylose, all these changes leaving the chain of the molecule intact. It is also possible that in the bundles of cellulose molecules lying parallel which make up the micelles of cellulose fibres, cross linkages between chains of different molecules in the form of ester linkages between a glucuronic acid unit and a hydroxyl group may exist. Such structures would defy detection owing to the methods used in isolation of celluloses but are nevertheless very plausible. Ordinary chemical criteria of homogeneity are, however, no longer applicable in dealing with natural polymers such as make up cell membranes.

Xylan has been shown by Haworth [7] to be constituted in an analogous way to cellulose :—



Its chain length has not yet been determined by methylation, but from the carboxyl equivalent it is estimated at 16 xylose units.

A similar structure has been suggested for the polymer of glucuronic acid, though a chemical proof has not yet been afforded.



The polysaccharide mannan in vegetable ivory is another such long chain molecule containing about 80 mannose units in the chain, but it is not certain whether it is homogeneously constituted of $\beta$-mannose units or whether it contains some $\alpha$-linkages.

It is quite probable that among the hemicelluloses, molecules may be found containing in a single chain, both glucose, glucuronic acid and xylose units: the hemicellulose from cotton-seed hulls contains both glucuronic acid and xylose.[8]

Bell's [9] work on wood cellulose shows that this cellulose is far less homogeneous than cotton cellulose, and may contain glucose chains of shorter length, and that there may be cross links between different molecules.

Cellulose occurs in lower as well as higher plants; thus the green alga *Valonia* has its cell wall built up of cellulose.

A number of workers have shown that the polysaccharide membrane synthesised by *Acetobacter xylinum* when grown in nutrient solutions containing glucose, fructose, mannitol, glycerol, etc., is a pure cellulose giving the typical X-ray pattern, and being convertible into 2 : 3 : 6-trimethylglucose.[10] The chain length is undetermined.

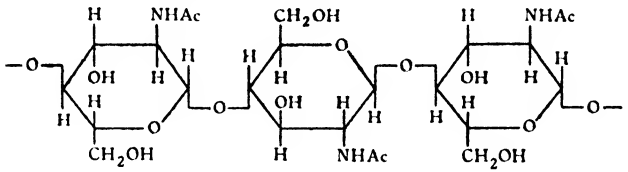
The same structure must be assigned to lichenin, which is an important component of the cell wall of lichens, and has been prepared notably from Island Moss, *Cetraria islandica*. Karrer was able to obtain from it on acetolysis cellobiose and also a triose lichenose, probably the same as cellotriose; since lichenin is water soluble, it must be considerably smaller in molecular size than cellulose.

The so-called animal cellulose of Tunicates is identical with plant

cellulose.[11] Tunicin, from *Polycarpa varians*, *Phallusia mamillata* and *Ascidia mentula*, gives the same X-ray pattern as ordinary cellulose.

Zechmeister [12] was able to prepare cellobiose, cellotriose, cellotetraose and cellohexaose from *Phallusia* tunicin.

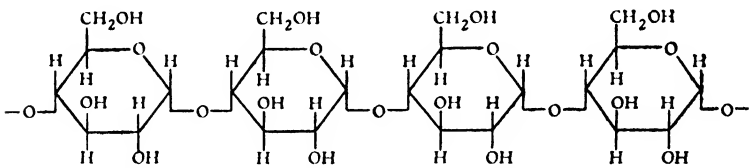
The cellulose type of linking of β-glucosidic units appears in the chitin forming the skeletal substances of *Crustaceæ* and *insecta*. Chitin is composed of β-N-acetylglucosamine units.



The chitin which occurs in the cell wall of fungi, but not in bacteria or higher plants, has been agreed by most authors to be identical with animal chitin.

## Starch.

The starch molecule is composed of a regular chain of α-glucopyranose units joined in glucosidic union at carbon 4. Starch is related to cellulose exactly as α-methylglucoside is to β-methylglucoside, or maltose to cellobiose.



Starch yields quantitatively glucose, or trimethyl starch 2 : 3 : 6-trimethylglucose on hydrolysis. The enzyme diastase converts starch almost quantitatively into the disaccharide maltose of proved structure. Chemical degradation also yields maltose, or starting with methyl starch the methylated oligosaccharides maltotriose and maltotetraose, in which α-glucosidic units are also present. No product of hydrolysis containing other forms of linkage has been found; a homogeneous chain of α-glucosidic units is also the only interpretation of the kinetics of the hydrolysis of starch, and is in harmony with the optical constants of starch and its breakdown products.[13]

The length of the starch molecule determined by the yield of tetramethylglucose is from 24 to 30 glucose units as a minimum.[14]

The same value is obtained both for the more soluble amylose fraction of the starch and the less soluble amylopectin fraction. The

chemical characteristics of these fractions appear to be identical in contradistinction to their physical properties.

An important point to be considered in connection with the structure of starch, is the alleged quantitative production of maltose from starch by the action of diastase, whereas probability considerations suggest the maximum possible yield produced by the haphazard attack of a hydrolytic agent at the glucosidic linkages would be 66 per cent. A simple explanation is that the enzyme works from one end of the molecule only.

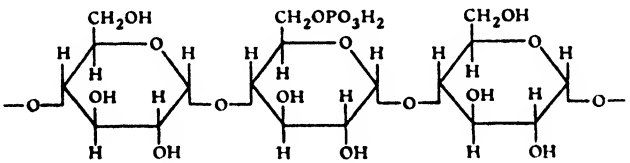
The nature of the terminal group at the aldehydic end of the starch molecule is undetermined: an oxidation or an anhydride structure have been discussed, since starch is almost completely non-reducing.

It is a striking fact that starch and cellulose, differing only in chain length and in their stereochemical configuration about the glucosidic link, should differ so remarkably in physical properties, in occurrence and in function.

Haworth has suggested that these differences are implicit in the configuration of the two substances. The $\beta$-glucosidic chains of cellulose can be stretched to straight extended chains, but the $\alpha$-glucosidic link does not permit of a stretched chain, but only a spiral. The starch molecules tend therefore to become entangled in one another, and it is this property which gives rise to the physical behaviour of starch solutions and to the slow retrogression of amylose to amylopectin.

Native starch also undoubtedly contains some phosphorus as phosphoric acid residues. (Wheat starch ·063; tapioca starch ·070; potato starch ·084 per cent. P.)

Posternak [15] has shown that from the degradation products of potato starch by the enzymes of bovine pancreas extract, phosphorus containing polysaccharides can be obtained in which the ratio of phosphorus to reducing groups is unity. From these polysaccharides on hydrolysis with sulphuric acid one can isolate the monophosphoric ester of a biose, which on further hydrolysis yields glucose-6-phosphoric ester identical with that obtained by Robison from yeast. The phosphoric acid residues are thus an integral part of the starch chain.



The studies of Haworth [16] have shown that animal starch, glycogen, has the same structure as vegetable starch, with, however, a shorter average minimum chain length of 12 glucose units. In keeping with the shorter length of its molecules, glycogen does not show the property of retrogression to an insoluble modification.

Galactogen, from *Helix*, is a galactose polymer having properties similar to glycogen.

Starches of lower molecular weight constitute the plant glycogen found in yeast, in fungi and in higher plants. Such products correspond to the dextrins produced in the early stages of the breakdown of starch with diastase.

The rather definite views on the structure of starch which are the result of chemical work, are not yet completely reconciled with views based on experiments on the action of enzymes on starch. Kuhn [17] made the discovery that in the hydrolysis of starch by enzymes either α- or β-maltose was produced, according to the nature of the amylase. Klinkenberg,[18] however, came to the conclusion that the enzymes acted on two different substrates: α-amylase acting on α-starches, comprising what is usually called amylopectin and also glycogen, and β-amylase acting on β-starch or amylose. According to him α- and β-starch are interconvertible, existing in the same proportions as α- and β-maltose in a maltose solution after mutarotation.

It was at one time considered that the production of β-maltose by the action of β-amylase spoke for the presence of β-glucosidic links in the starch molecule: this would be true if the maltose was cleaved from the middle of the starch molecule, unless it could be shown that a Walden inversion occurred on the enzyme cleavage of the α-glucosidic link. If, however, the maltose is produced from the end of the molecule, the β-maltose could be produced by β-amylase action on β-starch. The cleavage products would be β-maltose and a partly degraded α-starch which would have to revert to β-starch again before it could be hydrolysed by β-amylase. The action of α-maltase on α-starch would be exactly analogous.

The experimental evidence at present is hardly definite enough for an interpretation on these lines to be given.

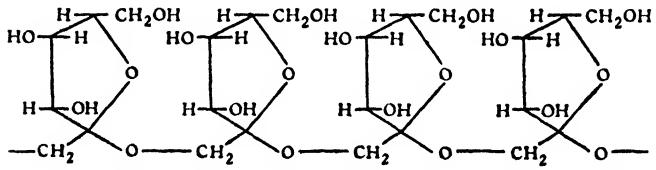
Waldschmidt-Leitz and Reichel [19] were able to degrade starch with a pancreatic amylase preparation to a mixture of dextrins, from which they were able to isolate in 10 per cent. yield a crystalline hexasaccharide.

## Inulin.

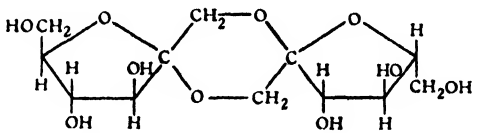
Inulin is a polysaccharide composed of fructose units, found in the underground storage organs of the *Compositae* and related families, where it replaces starch and accumulates during the winter months in tubers and rhizomes. It is also found together with starch in other parts of the plant.

Inulin is very easily hydrolysed by acids and is made up of $\gamma$-fructose units, trimethyl inulin being hydrolysed to 3 : 4 : 6-trimethyl-fructose.

The most probable structure is the following :—



Just as disaccharides are yielded on the breakdown of cellulose and starch, so a difructose is yielded by the breakdown of inulin. As this product is formed, it first presumably has an open-chain structure, but this reverts to the stable six-membered ring of the tricyclic difructose anhydride :—



Three forms of this anhydride exist, and three stereoisomeric forms are theoretically possible. It is produced in the hydrolysis of inulin itself either by acids or by inulinase and in the hydrolysis of acetyl and methyl inulin. There is no reason to suppose that the difructose anhydride pre-exists in inulin itself however, or that it is anything other than a reversion product.

The chain length of inulin determined by the yield of 1 : 3 : 4 : 6-tetramethylfructose of 3·7 per cent., is 30 fructofuranose units with a molecular weight of 5000.[20]

Haworth's belief is that the other end of the molecule is terminated by a reducing fructofuranose unit, accounting for the slight reducing properties of the best inulin preparations.

Some workers have consistently found a small amount of glucose to be yielded from inulin on hydrolysis, whatever its source, and have regarded it as an integral part of the molecule, rather than a

persistent impurity. Jackson and Macdonald [21] found 3·7 per cent. glucose, which gives a chain of 26 fructose units and 1 glucose in the molecule, about the same size found by Haworth.

Schlubach has also found glucose, and Pringsheim [22] has obtained a very pure inulin preparation, which has no reducing action. This preparation, after hydrolysis with inulase, yields 1·5 per cent. glucose, under conditions in which glucose is not formed secondarily from fructose. This result would give a chain length of about 70 units and a molecular weight of 11,000.

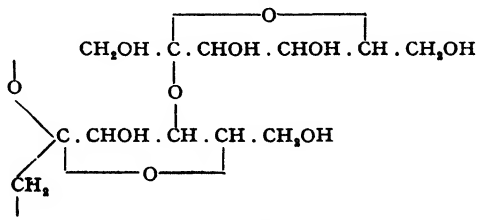
As a reconciliation it may be suggested that while inulin preparations isolated under certain conditions may consist only of fructose units, other preparations may consist of a fructose chain terminated at one end by a glucose unit, in the form of a sucrose residue. Inulin could be built up from sucrose in an analogous way to melezitose. A methylated inulin of this type should of course give 2 : 3 : 4 : 6-tetramethylglucose from the terminal group.

Tanret, the discoverer of inulin, and other workers have given evidence for the existence in *Compositae* of polyfructoses resembling inulin, but of less molecular complexity. Whether these substances really exist in the plant or whether they are degradation products analogous to the dextrins from starch remains undetermined. There have been isolated, however, a number of levulosans from monocotyledonous plants, notably the grasses, which consist mainly of fructofuranose units but yield some glucose in addition, and are of fairly small molecular dimensions.[23] It is premature to speculate on their structure until the methylation method has been applied.

A novel type of polysaccharide is presented by the polyfructose irisin,[24] conveniently obtained from the rhizomes of *Iris pseudoacorus*.

The rapidity of hydrolysis by acids speaks for it being wholly composed of γ-fructose units, it being also split by invertase.

The hydrolysis of methyl irisin yields an equimolecular mixture of 1 : 3 : 4 : 6-tetramethylfructose and a dimethylfructose which is probably 3 : 6-dimethylfructose. These facts are in harmony with the view that the following difructose is the polymerising unit,



and irisin may be looked upon as an inulin, containing an additional γ-fructose unit attached to every fructose unit in the main chain.

## Pectins.

Pectins consistently accompany cellulose as an integral part of the cell framework and the skeletal substance of plant tissues. They constitute up to one half of the dry weight of fleshy fruits, roots, leaves and green twigs, but occur only in traces in woody material, in which pectin is replaced by lignin.

The pectins are chiefly situated in the middle lamella, but are also found in the cellulose-containing cell wall; they are of similar importance as cementing materials between cells, to lignin in woody tissues.

Pectins are amorphous, colloidal substances characterised by giving mucilaginous solutions and forming gels, particularly in the presence of proper concentration of acids and cane sugar, the basis of jams and jellies.

Pectins from all sources appear to have the same general composition and are calcium and magnesium salts of a complex carbohydrate association. The primary pectin of the middle lamella is easily broken down to hydrato pectin, which is a mixture of 70 to 80 per cent. of pectic acid, and 30 to 20 per cent. of araban, a polymer of arabinose.

Pectic acid of the sugar-beet, from the proportions of the components which it furnishes on hydrolysis, can be formulated as a dimethoxy-diacetyl-arabino-galacto-tetragalacturonic acid. The pectic acid of the juice and flesh of fruits contains relatively more methyl alcohol and less arabinose, galactose and acetic acid.

Galacturonic acid is the major component of pectin, and by mild chemical treatment or by the action of certain enzymes on pectin a polygalacturonic acid can be obtained.

Ehrlich,[25] who has particularly studied pectin, considers the polygalacturonic acid to be a tetragalacturonic acid, with the four units joined in a circle, and that in pectin itself the other components are attached to this fundamental nucleus.

English pectin chemists have believed in a six-unit cyclic structure containing four molecules of galacturonic acid, one of galactose and one of arabinose.

The available analytical data can be used to support either theory, but both seem inherently improbable.

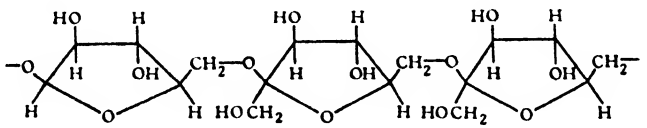
Work by Morell, Baur and Link[28] seems to establish that polygalacturonic acid chains of at least ten units are concerned in the pectin structure.

Polygalacturonic acid from citrus, on refluxing with methyl-alcoholic hydrogen chloride, yields in addition to α-methyl-*d*-galacturonide methyl ester an insoluble product regarded as the methyl glycoside of a polygalacturonide methyl ester. The ester methyl groups can be removed with dilute alkali which leaves intact the glycosidic methyl group. A methoxyl determination can then be used to determine the ratio between the terminal unit and the rest of the molecule. By this method a chain length, assuming a straight chain, of eight or ten galacturonic acid units was established, and corroborated by viscosity measurements by the Staudinger method.

The further development of pectin chemistry awaits systematic methylation experiments to determine the points of union of the component sugars.

## Polysaccharides of Bacteria.

Reference has been made above to the identity of the cellulose synthesised by *Acetobacter xylinum* with plant cellulose. Hibbert[26] and his co-workers have studied the polysaccharide levan produced by the action of *B. subtilis* and *B. Mesentericus*. The polysaccharide is composed wholly of fructose, and from the rapidity of hydrolysis it may be assumed to be made up of fructofuranose units. Hydrolysis of trimethyl levan yields 1 : 3 : 4-trimethylfructose. Levan is an isomer of inulin and may be formulated as a polymer of 2 : 6-anhydrofructose :—




The polysaccharide is produced only from sucrose and raffinose, but not from melezitose, nor from lactose, maltose, xylose, glucose or fructose. It appears that suitable substrates must contain an unsubstituted fructofuranose residue.

A polymerised glucose or dextran is produced by the action of *Leuconostoc mesenterioides* on sucrose, to a small extent from glucose, but not from other sugars.[27]

The polysaccharide membranes of bacteria have been investigated for the light they throw on the immunological reactions of pathogenic

bacteria. The polysaccharides of pneumococcus are discussed in detail in Chapter XVIII; similar specific polysaccharides have been isolated from a streptococcus, from *B. dysenteriæ*, paratyphoid bacilli, etc., and their chemical investigation is likely to develop owing to their importance in the study of immunity.

## REFERENCES TO CHAPTER XVII.


1. STAUDINGER, *Die Hochmolecularen organischen Verbindungen*, 1932.
2. MEYER AND MARK, *Der Aufbau der hochpolymeren Naturstoffe*, 1930.
3. KARRER, *Polymere Kohlehydrate*, 1925.
4. HAWORTH AND MACHEMER, J.C.S., 1932, 2270.
5. MARK, Trans. Faraday Soc., 1933, **29**, 41.
6. SCHMIDT, SIMSON AND SCHNEGG, Naturwiss., 1931, **19**, 1006.
7. HAWORTH, J.C.S., 1929, 1739; J.C.S., 1931, 2850.
8. ANDERSON AND KINSMANN, J.B.C., 1931, **94**, 39.
9. BELL, Biochem. J., 1932, **26**, 590.
10. HIBBERT AND TARR, Canadian J. Res., 1930, **4**, 372. HIBBERT AND BARSHA, Canadian J. Res., 1931, **5**, 580. MARK, C. 24, 500. GRASSMANN AND ZECHMEISTER, Naturwiss., 1932, **20**, 639.
11. SUTRA, Compt. rend., 1932, **195**, 181. KHOUVINE, CHAMPETIER AND SUTRA, Compt. rend., 1932, **194**, 208.
12. ZECHMEISTER AND TOTH, Z. Physiol. Chem., 1933, **215**, 267.
13. FREUDENBERG, *Tannin, Cellulose, Lignin*, 1933, p. 109.
14. HIRST, PLANT AND WILKINSON, J.C.S., 1932, 2375. HAWORTH, Trans. Faraday Soc., 1933, **29**, 3.
15. POSTERNAK, Compt. rend., 1933, **137**, 1157; 1934, **198**, 506.
16. HAWORTH AND PERCIVAL, J.C.S., 1931, 1342; J.C.S., 1932, 2277.
17. KUHN, Ann., 1925, **443**, 1.
18. KLINKENBERG, Z. Physiol. Chem., 1932, **209**, 253; **212**, 173.
19. WALDSCHMIDT-LEITZ AND REICHEL, Z. Physiol. Chem., 1934, **223**, 76.
20. HAWORTH, HIRST AND PERCIVAL, J.C.S., 1932, 2834. IRVINE, Chem. and Ind., 1932, 263.
21. JACKSON AND MACDONALD, Bur. Stand. J. Res., 1930, **5**, 1151.
22. OHLMEYER AND PRINGSHEIM, Ber., 1933, **66**, 1292.
23. SCHLUBACH AND ELSNER, Ber., 1929, **64**, 1493. DE CUGNAC, Ann. Sci. Naturelles, 1931, **13**, 1. COLIN AND NERFRON, Bull. Soc. Chim., 1931, **49**, 1542. COLIN AND CHAUDIN, Bull. Soc. Chim. biol., 1933, **15**, 402.
24. SCHLUBACH, KNOOP AND LIN, Ann., 1933, **504**, 30.
25. EHRLICH, Z. Angew. Chem., 1931, **44**, 463.
26. HIBBERT, TARR AND HARRISON, Canadian J. Res., 1930, **3**, 449. HIBBERT, TIPSON AND BRAUNS, Canadian J. Res., 1931, **4**, 221. HIBBERT AND BRAUNS, Canadian J. Res., 1931, **4**, 596. HIBBERT AND PERCIVAL, J.A.C.S., 1930, **52**, 3995.
27. TARR AND HIBBERT, Canadian J. Res., 1931, **5**, 414.
28. MORELL, BAUR AND LINK, J.B.C., 1934, **105**, 1.

# CHAPTER XVIII.

## THE RELATION BETWEEN CONFIGURATION AND BIOLOGICAL BEHAVIOUR.

It has long been known that the optical antipodes of a substance containing an asymmetric carbon atom behave very differently towards biological agents, such as yeasts, moulds, bacteria or enzymes. The mould, *Penicillium glaucum*, when grown in solutions of racemic acid, was found to assimilate only *d*-tartaric acid, leaving the *l*-tartaric acid untouched. It was supposed at first that the mould was unable to attack the *l*-tartaric acid; later investigations showed, however, that the mould ultimately destroys both antipodes, but attacks one at a very much greater rate than the other, and perhaps in a different manner.

From a given racemic substance it is possible to obtain sometimes the one and sometimes the other antipode by utilising appropriate organisms. For example, a preponderance of *d*-mandelic acid is obtained from *dl*-mandelic acid by the action of *Penicillium glaucum*, whereas when *Saccharomyces ellipsoideus* is used a preponderance of *l*-mandelic acid is obtained.

The relation between the stereochemical configuration of the sugar derivatives and their behaviour towards enzymes is now a commonplace, but it is no less remarkable on this account. It is the basis of many of the highly selective metabolic changes in plants and animals. In the domain of carbohydrates there are great opportunities for the illustration of specificity because of the abundant possibilities of isomerisation.

Glucose derivatives in which the terminal $C_1$ carbon atom is substituted exist in $\alpha$ and $\beta$ stereochemical modifications such as the $\alpha$- and $\beta$-methylglucosides. It was Emil Fischer [1] who discovered that these are hydrolysed by different specific enzymes. The $\alpha$-glucoside is hydrolysed by the maltase of yeast logically called $\alpha$-glucosidase; the $\beta$-glucoside is hydrolysed by the emulsin of almonds which contains $\beta$-glucosidase. Neither enzyme attacks the other glucoside.

Fischer studied the effect of a change in the configuration of the

groups attached to the other carbon atoms of the isomeric methyl-glucosides, and found that almost any alteration in their arrangement rendered the enzyme inactive. Consequently there arose the idea that the ability of an enzyme to effect hydrolysis depended on the exact configuration of the groups attached to every carbon atom of the substrate—the specificity was regarded as absolute.

In favour of this view were the facts that emulsin does not split β-methyl-*l*-glucoside,[2] nor did it appear to act on β-methyl-*d*-xyloside,[3] although this has the same configuration as the β-methylglucoside except for the groups on the terminal $C_6$ carbon atom. The corresponding rhamnoside, glucoheptoside and fructoside are also unaffected by emulsin.

Further investigations have shown that the alteration in structure of a glucoside from a six-membered pyranose ring to a five-membered furanose ring, also stops enzyme action : thus α-methylglucofuranoside is hydrolysed neither by maltase, emulsin nor invertase, and these three enzymes are also entirely without action on β-ethylglucofuranoside.[4]

The enzymes lactase, which hydrolyses milk sugar and β-methyl-galactoside, and invertase, which hydrolyses sucrose, were considered to be equally specific.

The exceptions to the conception of the absolute specificity of an enzyme were explained by postulating the presence of a second specific enzyme along with the first, a view which had been gaining ground until recently.

The positive action of almond emulsin on β-methylgalactoside[5] and on α-methyl-*l*-arabinoside,[6] likewise on α-methyl-*d*-mannoside,[7] was in each case attributed to the presence in the crude emulsin of another specific enzyme. On the other hand, the hydrolysis of β-methyl maltoside[8] in which the hydroxyl on $C_4$ is substituted by glucose, also that of β-methyl-*d*-isorhamnoside[9] were accepted as due to emulsin.

*The Enzymic Hydrolysis of β-glucosides.*—Emulsin is able to attack nearly all the natural β-glucosides though the speed of its action varies considerably according to the nature of the aglycone bound to the sugar, which may vary widely in constitution.

The following relative velocity constants for acid hydrolysis illustrate the variability of hydrolysis rate :—[10]

| | |
|---|---|
| Amygdalin . . | 85 |
| β-Methylglucoside . | 120 |
| Salicin . . . | 1233 |
| Arbutin . . | 2973 |
| Phloridzin . . | 7945 |

The variability of rates for enzyme hydrolysis is equally true, the following relative velocities being recorded by Willstätter and Oppenheimer :—[11]

| | |
|---|---|
| Phenyl-β-glucoside | 1 |
| Salicin . . | 4-6 |
| Helicin . . | 50-100 |

The relative rates of hydrolysis of a series of glycosides is by no means the same for enzymes as for acids. Thus while *o*-amino and *o*-acetyl-amino phenyl β-glucoside and β-galactosides are hydrolysed more rapidly by enzymes than the corresponding *p*-compounds, the latter are hydrolysed more rapidly by acids than the ortho-compounds.

Apart from other considerations, the enzymes are greatly influenced by the hydrogen ion concentration of the medium in which they act. When the aglycone contains a free acid group, a small quantity of emulsin is without action but in presence of "buffer" mixtures hydrolysis is effected. Amides and esters of the acids are more readily attacked than the free acids.

Similarly, the protection by acetylation of the amino group in the *o*- and *p*-aminophenyl β-glucosides and β-galactosides increases their rate of hydrolysis by enzymes. When the sugar residue is attached to a tertiary carbon atom as in linamarin, hydrolysis only takes place with great difficulty; other glucosides such as phloridzin, owing to low solubility, for long defied attempts to achieve their hydrolysis with emulsin, and conditions have so far not been secured for a successful hydrolysis of the anthocyanin β-glucosides with emulsin.

In general the glycosides of the phenols and phenol-carboxylic acids are more easily hydrolysed by enzymes than derivatives of the aliphatic alcohols, a fact which has led Helferich to make extensive use of the phenylglycosides as more sensitive test materials than the methylglycosides.

The idea that the various natural β-glucosides required many specific enzymes for their hydrolysis and that emulsin was a very complex mixture, has been abandoned in favour of the view that the same enzyme is always effective but the reaction is of very variable velocity.[12]

Helferich,[13] by comparison of preparations of emulsin of increasing purity, has advanced evidence to show that one and the same enzyme component which is conveniently called β-glucosidase, is capable of hydrolysing β-glucosides such as salicin and phenyl-β-*d*-glucoside, phenyl-β-*d*-maltoside, phenyl-β-*d*-isorhamnoside, phenyl-β-glucoside-6-bromohydrin, phenyl-β-*d*-galactoside, phenyl-β-*d*-xyloside, and phenyl-α-*l*-arabinoside.

The results suggest that lactase or β-galactosidase is identical with β-glucosidase, and the name lactase is consequently superfluous.

The following table shows the relative velocity of hydrolysis of these glycosides by the β-glucosidase :—

| Substrate. | Enzyme Value. | Time for 50 per cent. Hydrolysis. |
|---|---|---|
| Phenyl-β-*d*-isorhamnoside . . | 0·52 | 3·9 minutes |
| Phenyl-β-*d*-glucoside . . . | 0·29 | 6·9 ,, |
| Phenyl-β-*d*-maltoside . . . | 0·15 | 13 ,, |
| Phenyl-β-*d*-galactoside . . . | 0·035 | 57 ,, |
| Phenyl-α-*l*-arabinoside . . . | 0·022 | 91 ,, |
| Phenyl-β-*d*-glucoside-6-bromohydrin | 0·003 | 670 ,, |
| Phenyl-β-*d*-xyloside . . . | 0·002 | 1,000 ,, |

Helferich finds that phenyl-β-*d*-isorhamnoside is actually hydrolysed by emulsin more rapidly than is any other phenylglycoside.

The ratio between the quickest and the slowest hydrolysis in the table is 260 to 1. It is of interest to compare the enzyme value of phenyl-β-*d*-xyloside with those of glycosides, which are at present considered as not hydrolysable by any component of emulsin.

| Substrate. | Enzyme Value. |
|---|---|
| Phenyl-α-*d*-glucoside . . . | ·000002 |
| Phenyl-β-*d*-glucoside-3-methyl-ether | ·00005 |
| Phenyl-β-*d*-mannoside . . . | ·00005 |
| Phenyl-α-*l*-rhamnoside . . . | ·00001 |
| Phenyl-β-*d*-fructoside . . . | ·00005 |

The enzyme value of a glycoside which is classed as " unhydrolysable " is one-fortieth of the value of the slowest hydrolysed glycoside. The very slow apparent hydrolysis of these " unhydrolysable " glycosides may be due to the spontaneous hydrolysis at $p_H$ 5·0.

When the formulæ of these compounds are compared, it is clear that changes in the configuration of the radicals on carbons $C_6$ and $C_4$ are possible without rendering the enzyme inoperative, but not those on carbons $C_1$ and $C_2$, nor in all probability on $C_3$.

While the groups on $C_4$ may be inverted, and $C_6$ may be substituted with small groups without loss of specificity, substitution of $C_4$ or $C_6$ with more bulky groups is sufficient to make β-glucosidase inactive. Thus 6-methyl- and 4- and 6-toluenesulphonyl phenyl-β-glucosides are not split.[15]

When the $C_6$ substituent is a second molecule of glucose the specificity problem is less clear. β-Gentiobiosides are split finally to two molecules of glucose and the aglucone, but it seems established that

the enzyme first attacks the gentiobiose linkage, splitting the compound to glucose and a simple glucoside which is then further attacked.[16]

There is in emulsin a ferment which splits β-maltosides, but its activity is not considered to run parallel with the β-glucosidase activity. Phenyl-β-maltoside is hardly split any faster than methyl-β-maltoside, and purification increases the β-glucosidase activity more rapidly than the β-maltoside value. The split products are maltose and the aglucone; there is thus an enzyme whose action is not stopped by substitution of $C_4$ by a second glucose molecule, but it may not be identical with β-glucosidase.[17]

The grouping possessed in common by all the hydrolysable substrates is the *trans* configuration of the substituents of carbons $C_1$ and $C_2$ in conjunction with the aldopyranose ring. Helferich suggests *trans* aldopyranosidase as an alternative name for the enzyme.

Whereas formerly the idea that enzyme and substrate must be compatible along the whole length of the molecule gave rise to the key and lock simile of Emil Fischer, it is now necessary to modify this simile by saying that there is a master key which is able to turn several locks; provided that the substrates are identical with respect to the configuration of the first three carbon atoms and the pyranose ring, other variations are permissible without the loss of hydrolysability by β-glucosidase.

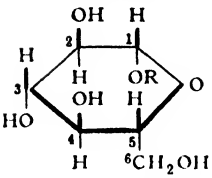
Crude emulsin contains a second component which is capable of hydrolysing phenyl-α-*d*-galactoside and phenyl-β-*l*-arabinoside. These glycosides contain in common a *cis* configuration for carbons 1 and 2 and an aldopyranose ring. The enzyme is probably identical with the melibiase in bottom fermentation yeast which splits melibiose, and may be called α-galactosidase.

Finally, crude emulsin hydrolyses phenyl-α-*d*-mannoside and phenyl-α-2-desoxyglucoside;[18] the attack on these two last substrates almost certainly being due to a third separate enzyme α-mannosidase. A further component is that which hydrolyses chitin.

Helferich[14] has adduced evidence that after emulsin has been partially inactivated by exposure to ultra-violet light its action on β-glucoside and β-galactoside remains parallel, though that towards α-*d*-mannoside is different, indicating a distinct enzyme for this hydrolysis. Partial destruction of the enzyme with formaldehyde even indicates a difference between the action on glucoside and galactoside, but this requires confirmation.

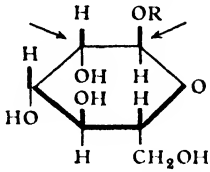
## Selectivity of Emulsin.*

*Hydrolysed.*

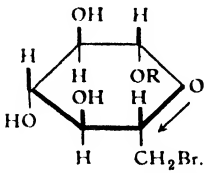


(trans) β-*d*-glucoside.

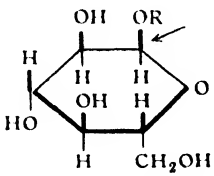
*Not hydrolysed.*



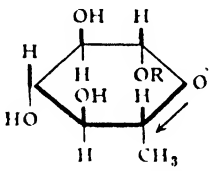
(trans) α-*d*-mannoside.



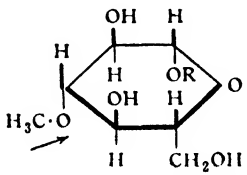
(trans) β-*d*-glucoside-6-bromohydrin.



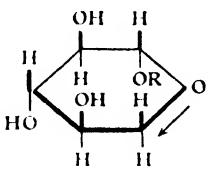
(cis) α-*d*-glucoside.



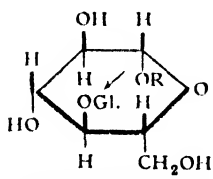
(trans) β-*d*-isorhamnoside.



(trans) β-*d*-glucoside-3-methyl ether.



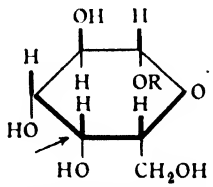
(trans) β-*d*-xyloside.



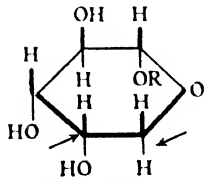
(trans) β-*d*-maltoside.

* The arrows indicate where the original β-glucose molecule has been modified.

*Hydrolysed.*

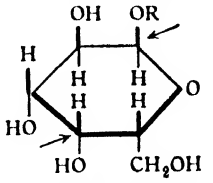



(trans) *β-d*-galactoside.

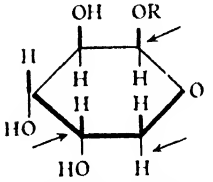



(trans) *α-l*-arabinoside.

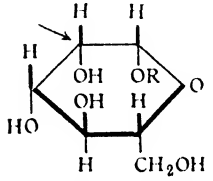
*Not hydrolysed.*



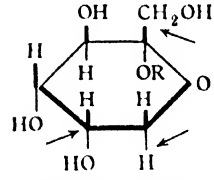

(cis) *α-d*-galactoside.



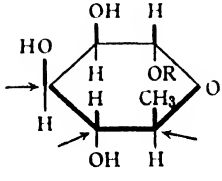

(cis) *β-l*-arabinoside.




(cis) *β-d*-mannoside.




(trans) *β-d*-fructoside.




(trans) *α-l*-rhamnoside.

*Maltase.*—The specificity of maltase (α-glucosidase) has been less fully investigated than that of the β-glucosidase of emulsin.

It splits maltose, sucrose, melezitose and maltosazone in virtue of their being α-glucosides, α-methylglucoside and α-glucosides in general, but apparently no glucoside in which the α-glucose residue has been altered or substituted.

Thus it is without action on α-methylgentiobioside where a second glucose molecule is attached to the α-methylglucoside at $C_6$,[19] similarly it does not act on phenyl-α-lactoside and phenyl-α-cellobioside.

It likewise has no action on α-methylglucoside-6-chloro and -6-bromohydrin, on anhydro-α-methylglucoside, on α-methyl-*d*-isorhamnoside and on α-methylglucoside-6-methyl ether.[20] It is also without action on α-methylglucofuranoside.[21]

α-Phenylglucoside is much more readily hydrolysed than α-methylglucoside by maltase, and should be used as the test substrate for this enzyme. For example, the small concentration of maltase which is in barley malt is almost without action on α-methylglucoside but it hydrolyses α-phenylglucoside.

## The Enzyme Hydrolysis of Sucrose.

Modern views of enzyme specificity no longer support the once held theory that enzymes can be specific for a disaccharide as a whole, as invertase was thought to be for sucrose, but tend to follow Weidenhagen [22] in speaking only of glycosidases which are specific for the glycosidic sugar only, and whose action is independent of the other constituent of the molecule which may be a sugar or an aglycone.

Sucrose is both an α-glucoside and a β-fructofuranoside, and is therefore hydrolysed by two different enzymes, maltase and invertase.

Invertase hydrolyses β-fructofuranosides, sucrose and those oligosaccharides which contain a sucrose residue in their molecule, in which the fructose is unsubstituted other than at the reducing group.

Thus it hydrolyses—

Sucrose = glucose < > fructose
Raffinose = galactose < glucose < > fructose
Gentianose = glucose < glucose < > fructose
Stachyose = galactose < galactose < glucose < > fructose

at the glucose < > fructose junctions, but is without action on melezitose.

Glucose < > fructose > glucose

in which the fructose is in the middle of the molecule.

Yeast contains both invertase (β-fructofuranosidase) and maltase (α-glucosidase), but the enzymes have a different optimum *p*H for their action on sucrose. Maltase is most active at *p*H 6·7 and is inactive at *p*H 4·5, the optimum for invertase. These differences allow the amounts of the different enzymes to be estimated in varieties of yeasts or in fungi.

Weidenhagen [23] has separated the α-glucosidase from the invertase in yeast by the Willstätter adsorption methods, and has shown that it hydrolyses sucrose at about half the rate of maltose, but does not hydrolyse raffinose.

From time to time it has been considered that disaccharides of the reducing type could be hydrolysed by two enzymes, one compatible with the glycosidic component, the other with the other component. The experimental basis for these examples has fallen to the ground.[24]

The specificity of enzymes has been investigated by following the alteration, if any, in the rate of hydrolysis of a glycoside or a disaccharide when various sugars are added to the solution.

The hydrolysis of maltose by maltase [25] takes place more slowly in the presence of glucose, while mannose, galactose, fructose, arabinose and xylose are without action, proving that the effect is due to the special configuration of glucose and not to concentration changes.

Inhibition is due to the reversal of the hydrolysis, the slowing up of hydrolysis being also due to the cleavage products having an affinity with the enzyme surface.

There occurs a distribution of the enzyme between the substrate and the cleavage or added product.[26] It is possible to calculate from experimental data the affinity constant of invertase to various sugars which is of the relative order of 60 for sucrose, 17 for fructose, 11 for glucose and 0 for lactose.

Inhibition experiments have not thrown the light on the structure of enzymes which it was at one time thought they might, owing to the complication that the added sugars mutarotate giving a mixture of α- and β-forms, while fructose in solution is fructopyranose and not fructofuranose as it is in sucrose.

Yeast invertase is inhibited by fructose and β-glucose, but usually not by α-glucose. This is because it contains mainly γ-fructosidase and relatively little α-glucosidase. The invertase of takadiastase (*Aspergillus oryzæ*) is strongly inhibited by α-glucose, as is that of *P. glaucum*, which is not inhibited by fructose. This is in keeping with the finding that the sucrose splitting enzyme of moulds is an α-glucosidase.

## Enzymic Splitting of Polysaccharides.

It is an interesting question as to whether the polysaccharides composed of a β-glucosidic type of unit, such as cellulose, lichenin, tunicin, mannan, xylan and chitin, are hydrolysed by a common enzyme, or whether they require a specific catalyst.

Cellulases are not found in the digestive tract of higher vertebrates, but those of crustaceæ, of insect larvæ and of molluscs are particularly active. The activity of the cellulases in higher plants, such as those in seeds, is also low, but the mould fungi have by contrast a great power for splitting polysaccharides, such as the cellulose type and pectin and inulin.

It appears that mannanase, xylanase, and inulinase are different specific enzymes, and cellulase is probably different from the lichenase contained in mould fungi.[27]

Fungal cellulase has the same activity towards the animal cellulose, tunicin, from *Phallusia mammilaris*, as it has towards vegetable cellulose, confirming their identity established by chemical methods.

A series of substrates of known structure is provided by the β-glucosides, cellobiose, the other crystalline oligosaccharides derived from cellulose, the cellodextrins, and cellulose.

The β-glucosidase of emulsin which splits β-glucosides is clearly not identical with the cellulase from mould fungi. Fungal cellulase (*from A. oryzæ*) is far more active on cellulose than crude emulsin, i.e. some two to three thousand times for preparations which have equal activity to β-glucosidase when measured towards salicin.

From the crude fungal enzyme mixture it is possible by adsorption on aluminium metahydroxide to separate cellulase from a cellobiase.[28] It is established that cellulase hydrolyses cellulose and the cellodextrins down to a molecular weight of about 1000; cellobiase hydrolyses cellobiose (m.wt. 342), cellotriose (504), cellotetrose (666), cellohexaose (990), but not cellodextrin (1500). It is also without action on most β-glucosides.

It is possible thus to distinguish between polysaccharase, oligosaccharase and β-glucosidase. The former acts on large molecules which, so to speak, are all middle with no ends, and attacks at about every tenth glucosidic linkage in the long chain. The oligosaccharase acts on the shorter molecules which the polysaccharase produces.

β-glucosidase hydrolyses all β-glucosides and cellobiose.

A parallel is provided by the behaviour of inulase (from *Aspergillus*) and β-fructosidase (from yeast) towards inulin and sucrose.[29] The

inulase is relatively much more active towards inulin, whereas invertase hydrolyses sucrose at over five thousand times the rate at which it hydrolyses inulin.

The enzyme degradation of starch also appears to be effected in steps, by a series of enzymes.

These findings are somewhat in conflict with Weidenhagen's generalisation that the specificity or otherwise of an enzyme is only determined by the sugar component which is exerting the glycosidic function in the substrate.

It is questionable how far it can be true to say that even a $\beta$-glucosidase from two different plants is the same in both. The modern view is that enzymes consist of active groups distributed throughout a high molecular carrier, usually a protein : the carrier is to be expected to vary from one plant to another and, therefore, the enzyme cannot be said to be identical, even if the active group is identical, which will have the practical result of making the enzymes appear identical in activity.

It is possible too, that in comparing the $\beta$-glucosidase activity of higher plants with that of mould fungi, one is comparing enzymes which it is true may exert the same function, but differ in chemical make-up both as to the carrier and to the active group.

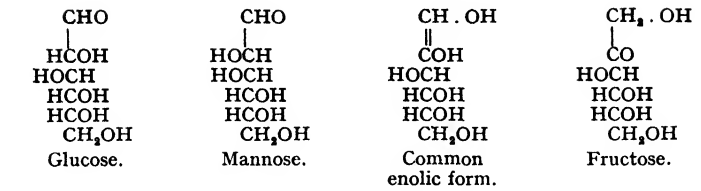
But even these useful considerations cannot explain away the established existence of a cellulase and a cellobiase of limited specificity.

## Fermentation.

The investigation of the behaviour of all the known hexoses, either found in nature or prepared in the laboratory, towards yeasts has shown that only four are fermented, viz. the *d*-forms of glucose, mannose, galactose and fructose, all of which are natural products.

When the behaviour of different species of yeasts towards these natural hexoses is studied, it is found without a single exception that any species of yeasts which ferments any one of the three hexoses—glucose, mannose and fructose—likewise ferments all three of them and with approximately the same readiness. The study of the kinetics of the three fermentation reactions confirms their similarity, and they have the same temperature coefficient. Everything, in fact, points to the mechanism involved in the fermentation of glucose, mannose or fructose being the same in each instance.

An enolic form common to all three hexoses has been assumed to act as an intermediate substance in the transformation.[30]



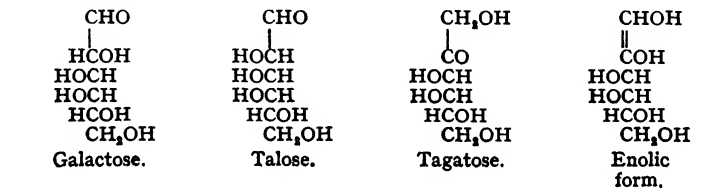

It is supposed that the first process in fermentation is the conversion of the sugar into the enolic form by means of an enzymic mechanism in which the sugar phosphate is concerned. The three fermentable hexoses yield the same enolic form, but possibly it is formed at different rates according to the sugar; the subsequent breakdown of the molecule is the same for each of the three hexoses.

This view is in harmony with the discovery by Harden and Young that the first stage in the fermentation of glucose by zymase is the formation of hexose phosphate $C_6H_{10}O_4(H_2PO_4)_2$. Glucose, mannose and fructose give rise to the same hexose phosphate: when this is hydrolysed fructofuranose is obtained.

No difference can be detected in the rate of fermentation of $\alpha$- and $\beta$-glucose under conditions in which fermentation is quicker than the setting up of equilibrium between the two forms.

Substances so closely related to glucose as the methylglucosides, glucosone, gluconic acid and ethylgluconate, are, without exception, unfermentable: none of them can give the enol form of glucose, and no action takes place since the formation of hexose phosphate is impossible.

The behaviour of galactose is altogether different. It is fermented with much greater difficulty than glucose. Very many yeasts are quite without action on galactose. The temperature coefficient of the fermentation of galactose is different from the value found for glucose. These facts suggest that galactose is fermented by a different mechanism, that a different enzyme is concerned perhaps in causing enolisation and phosphate formation, which is less widely distributed in yeasts. None the less the two phenomena must be very closely allied. No yeast is known capable of fermenting galactose but not fermenting glucose.



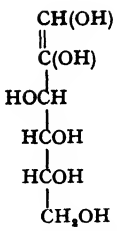
It is remarkable that neither talose nor tagatose is fermented by any yeast whose action towards them has at present been investigated. Yet in talose the position of the two upper hydroxyl groups is the same as that in mannose, and the lower three hydroxyls occupy the same positions as they do in galactose. Obviously, for it to be fermentable the configuration of the hexose has to be correct as a whole, the fact that single hydroxyl groups occupy the same positions as they do in fermentable hexoses being of no moment.

The facts described can only be explained on the assumption that there is the very closest relationship between the configuration of a fermentable hexose and the enzymes which cause fermentation. This hypothesis receives confirmation which is little short of absolute when the behaviour of the sugars other than the hexoses is considered. No pentose, either natural or synthetical, is fermentable by yeast. None of the synthetic tetrose, heptose or octose carbohydrates are fermentable.

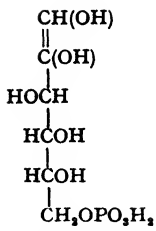
The only fermentable sugars, other than the four hexoses, are a *nonose*, prepared by the cyanhydrin method from mannose, and a ketotriose, *dihydroxyacetone*. The fermentability of "glycerose"—a mixture of glyceric aldehyde and dihydroxyacetone—was long a matter of controversy; Bertrand, however, showed that pure dihydroxyacetone was fermented by very active yeasts and this has been repeatedly confirmed. Lebedeff and Griaznow [31] have proved that dihydroxyacetone is first converted into hexose phosphate and that the synthesis of a hexose is preliminary to fermentation.

Harden pictures the following course of events during fermentation. The hexose is first converted into the enolic form, the three fermentable hexoses undergoing this change at different rates. The enolic form is then esterified at $C_6$ by a phosphatase—Robison supposes that $C_1$ is first esterified and that the phosphoric acid group wanders to the neighbouring $C_6$. On further esterification a 1 : 6 diphosphate is produced. These esters revert to the ring form: the monophosphoric ester can give a mixture of all three sugars, while the diphosphoric ester can only be derived from fructofuranose. The diphosphoric ester is more readily enolised than are the hexoses. Both esters when hydrolysed yield mixtures of aldose and ketose sugars.

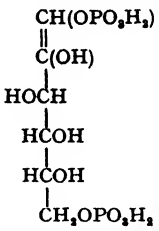
Simultaneously with the esterification an induced reaction occurs in which the carbon chain of another molecule of sugar, or more probably of the enol, is ruptured to yield two 3-carbon atom molecules, one molecule of sugar being broken up for every two phosphoric groups esterified.



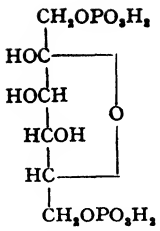
Enolic form common to glucose, mannose, fructose.



6-phosphoric ester of enol.



1 : 6 diphosphoric ester of enol.



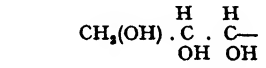
1 : 6 diphosphoric ester of fructofuranose.

## Oxidation.

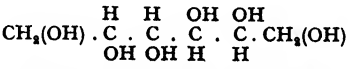
The influence of configuration is well illustrated in the behaviour of carbohydrates towards oxidising bacteria. The *Bacterium xylinum* (Adrian Brown), or sorbose bacterium, as it was termed by Bertrand, oxidises aldoses to the corresponding monobasic acids. Gluconic acid is formed from glucose, galactonic acid from galactose; xylose and arabinose yield xylonic and arabonic acids. In all these the —CHO group is oxidised to —$CO_2H$ by the agency of the bacterium.

In alcohols the sorbose bacteria oxidise —CH(OH)— to —CO—. Thus mannitol forms fructose; sorbitol yields sorbose; erythritol, *l*-arabitol, α-glucoheptitol and persitol are oxidised to the corresponding ketoses, and glycerol gives dihydroxyacetone. The bacterium has no action, however, on ethylene glycol, dulcitol or xylitol.

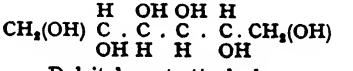
An examination of the formulæ of these alcohols shows that the —CH(OH)—group oxidised to —CO— is next to a —$CH_2$(OH) group and also to a second —CH(OH)— group, with respect to the OH group of which its OH group must have a *cis* configuration in the projection formula; in other words, the compound must contain the grouping [32]



Consideration of the formulæ of mannitol and dulcitol will help to make this clear :—

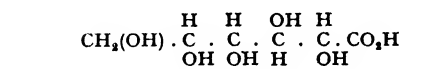


**Mannitol—converted into fructose.**

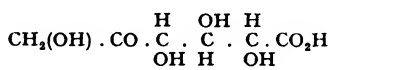


**Dulcitol—not attacked.**

Gluconic acid contains the sensitive grouping. Accordingly, it is further oxidised by the bacterium to 5-keto-gluconic acid.



Gluconic acid.



Ketogluconic acid.

These oxidising bacteria appear to depend on a particular configuration of a section of the molecule only for their action to take place. However, Votoček, Valentin and Rac [33] found that sorbose bacteria had no action on *l*-rhamnitol nor on α- or β-rhamnohexitol, although both of these contain the configuration required by Bertrand's rule.

They conclude that the oxidising action is determined not only by stereochemical configuration but by the homologous series to which the alcohol belongs.

A study of a number of well differentiated strains of *Acetobacteria* by Hermann and Neuschul [34] revealed that all were capable of oxidising ethyl and propyl alcohol to acetic and propionic acids; all but one oxidised glucose to gluconic acid; and all but three oxidised arabinose and galactose to arabonic and galactonic acids.

In addition to *B. xylinum*, the following were able to form the ketone group from compounds of appropriate configuration (i.e. glycerol, erythritol, mannitol, sorbitol and gluconic acid): *B. gluconicum*, *xylinoides*, *orleanense*, *aceti* (Hansen).

The remaining seven bacteria studied were aketogenic.

Another bacterial oxidation is the conversion of mannitol to mannose or dulcitol to galactose by means of *B. mesentericus* studied by Péré,[35] in which a $CH_2OH$ group is oxidised to CHO.

Many bacteria act upon mannitol which are without action on dulcitol. Harden found this to be true for *Bacillus coli communis*, which is of interest also since it produces twice as much alcohol from mannitol as from glucose. This difference is ascribed to the presence of the group $CH_2(OH) . CH(OH)$— which is contained once only in glucose but twice in mannitol.

The oxidative fermentation of sugars by mould fungi has been fully studied of recent years. Different moulds exhibit marked specificity in their power of attacking different sugars, and in the diverse products which occur in their culture solutions.

From glucose may be produced gluconic, oxalic, citric, succinic, fumaric, malic and kojic acids, ethyl alcohol, acetaldehyde, ethyl acetate, mannitol and glycerol. Besides these common products occur complex substances, fats, sterols, citromycetin, citrinin and

other aromatic compounds, and polysaccharides, such as were found by Raistrick and Rintoul [36] in their comprehensive studies of 240 different species of lower fungi.

The alteration in cultural conditions with the formation of certain acids and the suppression of others, by the same fungus species, and the study of different acids produced by different fungi has made it possible to accept a uniform scheme of sugar metabolism leading to the formation of the acids in mould fungi.

The increased yield of desired product obtained by adjusting the culture medium has made possible the use of these moulds for industrial purposes in the production of gluconic and citric acids.

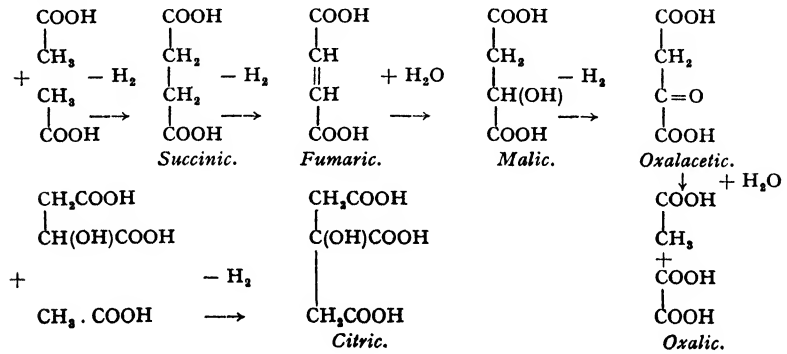
*Aspergillus niger*, *Citromyces glauber*, and *Penicillium glaucum* and a series of related fungi are able to form gluconic acid together with citric acid. Low activity favours the production of gluconic, and high of citric acid.[37]

Bernhauer and Siebenäuger [38] have shown that two races of *Aspergillus niger* are capable of forming citric acid from ethyl alcohol, and from acetic acid in 16 per cent. yield: similarly, Butkewitsch and Feodoroff [39] find that oxalic acid can be produced by *Mucor stolonifer* from acetates, and the genus *Rhizopus* can form fumaric and succinic acids from acetates.[40]

Chrzaszcz and Tiukow [41] found an unnamed *Penicillium* to produce in sodium potassium acetate media, varying amounts of succinic, fumaric, oxalic, *l*-malic and citric acids.

These results support the parallelism of the sugar metabolism of these moulds with that of yeast: after breakdown of sugar to pyruvic acid, acetaldehyde and ethyl alcohol, and oxidation to acetic acid, the latter is converted to succinic, fumaric, malic, citric and oxalic acids.

The following scheme represents the probable mechanism involved:—[42]



Calcium gluconate is converted by a species of *Aspergillus niger* into citric acid and smaller amounts of oxalic acid, whereas oxalic acid is almost exclusively produced by *A. niger cinnamomeus.*[43]

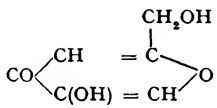
The oxidation of glucose to *d*-glucuronic acid by moulds has been reported by Wünschendorff and Kiliani,[44] but lacks confirmation.

The oxidation of glucose at both ends of the molecule to saccharic acid by means of *A. niger* has been studied by Challenger.[45]

It is of interest that while *d*-gluconic and *d*-mannonic acids are produced by *Penicillium purpurogenum* from *d*-glucose and *d*-mannose respectively, no acid could be isolated from *d*-galactose.[46]

Xylose is a better substrate for the formation of citric acid by mould fungi than arabinose.[47] It is supposed that the pentoses are first degraded to a $C_3$ compound which forms a $C_6$ sugar and that this is converted to citric acid. Both pentoses can serve as a source for the fermentative production of oxalic acid.

It is a striking fact that lactic acid has never been reported as a mould metabolic product. The formation of the pyrone derivative, a kojic acid



is at first sight an indication of a direct derivation from the pyrane nucleus in glucose. Challenger,[48] however, has shown that kojic acid is formed by the mould from arabinose and xylose and in best yield from dihydroxyacetone, and believes that it is built up from a triose.

The occurrence of the various sugar alcohols in plants has evidently some relation to their metabolism.

By floating detached leaves, which have been deprived of their starch by keeping them in the dark, on nutrient solution, it is possible to determine which substances can occasion the formation of starch. The application of this method to the carbohydrate alcohols affords an excellent illustration of the influence of configuration on the biological properties. Plants which normally contain alcohols can utilise these and also glycerol to form starch ; thus the *Oleaceæ* utilise mannitol, *Ligustrum* and *Cheiranthus* make use of dulcitol. Treboux has shown that the Rosaceæ are able to produce starch from sorbitol, the production being more vigorous than from carbohydrates or from glycerol, but they are quite unable to utilise mannitol or dulcitol. The leaves of *Adonis vernalis* are able to convert adonitol into starch, but can make use of no other carbohydrate alcohols.

The specific behaviour of the various sugars towards bacteria affords abundant examples of the influence of configuration.

Thus Quastel's [49] researches with dehydrogenase, in which the activity of a sugar as a donator of hydrogen with respect to *B. coli communis* is measured, show that arabinose is almost inactive, xylose has a reducing coefficient of 20, galactose of 200, glucose, fructose and mannitol of 5000; whereas dulcitol is without action.

It is clear that the dehydrogenase deals with a particular type of molecular configuration only.

In all cases where fermentation of sugar is known to occur an activation of the molecule with subsequent reducing action on methylene blue takes place. Sugars which are not activated are not fermented.[50]

Of the four sugars, glucose, fructose, mannose, galactose, some bacteria ferment all four, others have no action on galactose. One, *B. Morgani*, ferments glucose only, another, *B. Proteus*, ferments glucose and galactose only. A smaller number of bacteria are able to ferment xylose.

A comparison of the fermentation of *d*- and *l*-arabinose by bacteria has been made.[51]

The common *l*-arabinose is fermented rapidly by many types; the same organisms ferment the *d*-form with difficulty if at all and only at a late stage. Three types of *Proteus vulgaris* attacked *d*-arabinose and not the *l*-form.

## Specific Carbohydrates and Immunology.

Certain carbohydrates are of fundamental importance in the obscure immunological reactions: indeed, it is through their study that great progress is being made in this subject.

The most complete studies have been made so far on pneumococcus, an organism which is found in at least three distinct strains, known as types I., II. and III.

Pneumococcus is an encapsulated organism, and Avery and Heidelberger,[52] to whom we owe most of the advance in this field, have produced evidence that the ectoplasmic layer of the pneumococcus cell is composed of carbohydrate material which is identical in all its biological characters with the type specific substance of pneumococcus. The endoplasm consists of a protein which is species and not type specific: it is possessed in common by all pneumococci, whilst the carbohydrate is chemically distinct and serologically specific for each

of the three fixed types. In short, it is type specific but not antigenic. The chemical characteristics of the specific carbohydrates have been established as those of polysaccharides yielding on hydrolysis their components as follows :—

| | | $[\alpha]_D$. |
|---|---|---|
| Type | I. = galacturonic acid + an amino sugar | + 300° |
| ,, | II. = glucose + glucuronic acid | + 74° |
| ,, | III. = glucose + glucuronic acid | − 33° |

Type III. polysaccharide appears from its properties to be a polymer of an aldobionic acid, and has a molecular weight of about 4000.[53]

Type I. polysaccharide has one half of its nitrogen liberated with nitrous acid. Recently it has been found that when isolated under milder conditions this polysaccharide bears one acetyl group to every four monosaccharide units.[54]

Other micro-organisms are being found to contain similar specific carbohydrates. Thus a polysaccharide from Friedländer's bacillus which also has a voluminous capsule [55] $[\alpha]_D$ + 100° contains glucose, glucuronic acid and perhaps another sugar. It is markedly similar biologically in cross precipitation tests to the pneumococcus Type II., sufficiently so indeed to suggest that both substances contain the same configuration of a part of their molecule.

By partial hydrolysis of gum-arabic a polysaccharide of similar specific activity to the soluble substances produced by pneumococcus is obtained which on further hydrolysis yields a crystalline aldobionic acid.[56] Challinor, Haworth and Hirst [57] have established this as 6-galactopyranose glucuronic acid.

A striking confirmation of the views which have been developed on the rôle of the specific polysaccharides of pneumococcus is provided by the work of Dubos and Avery.[58]

These workers isolated a bacterium from peat soil which is able to hydrolyse the type III. polysaccharide after it has been cultivated in media containing the polysaccharide : the enzyme responsible can be extracted by autolysis from the bacterial cells. It proved possible to cure mice, rabbits and finally monkeys [59] which were infected with type III. pneumonia, by injection of the enzyme. The enzyme is able to hydrolyse the polysaccharide capsule rendering the bacteria susceptible to phagocytosis by the cells of the body, and thus exerts its curative effect.

The tubercle bacilli have been proved to contain similarly specific sugars. Thus, when they are propagated in a sugar-free medium a polysaccharide composed of *d*-mannose and *d*-arabinose is obtained,[60]

and the same two carbohydrate units are present as in the polysaccharide which can be isolated from Long's synthetic media, in which tubercle bacilli have grown. These sugars, together with galactose and inositol, are present in the polysaccharide extracted by toluene along with the lipoids of the entire bacilli.[62] The occurrence of *d*-arabinose instead of the *l*-arabinose of plants in these compounds is noteworthy.

The further attack on the problem of the specificity of immune reactions has been made by coupling known sugar residues with proteins of ascertainable antigenic properties. Goebel and Avery [63] have synthesised *p*-aminophenol β-glucoside and β-galactoside and coupled them, after diazotisation, with globulins and albumins. The four compounds obtained are called gluco- or galacto-globulins and albumins: in the globulin compounds, the glucoprotein contained 17 per cent. and the galactoprotein 10 per cent. of reducing sugar. Avery and Goebel found that when two different sugars are bound to the same protein the resulting antigens exhibit distinct specificity. When the same sugar is conjugated with two chemically different and serologically distinct proteins, both substances acquire a common specificity. It is the character of the carbohydrate component that determines the newly acquired specificity of the synthetic sugar protein, and it is seen how the difference between glucose and galactose in molecular configuration is enough to orient protein specificity: though in view of the high proportion of sugar in the molecule this is not surprising. The sugar compounds unattached to proteins are non-antigenic, but specifically reactive. A study of the active and passive anaphylaxis induced by these sugar proteins has also been made.[64] The serum of rabbits immunised with glucoglobulin sensitises guinea-pigs to glucoalbumin, so that they exhibit typical anaphylactic shock.

In a further research Goebel and Avery [65] synthesised the *p*-aminobenzyl ether of the specific polysaccharide type III. pneumococcus and coupled this, after diazotisation, with globulin. On testing the synthetic antigen on rabbits, it was found to show the type specific response, which neither one of its constituents incited when injected singly into the animal.

In this instance the protein is of remote biological origin and the carbohydrate alone is not antigenic, yet proof is afforded that an effective active immunity can be developed in which the only antibacterial antisubstances formed are those directed against the capsular polysaccharide.

It has also been shown [66] that not only is it possible to distinguish immunologically between glucose and galactose, but also between the two isomeric $\alpha$- and $\beta$-glucosides of the same sugar. There is a difference between the protein complexes of *p*-aminophenyl $\alpha$- and $\beta$-glucosides.

## REFERENCES TO CHAPTER XVIII.


1. Fischer, Ber., 1894, **27**, 2985.
2. Fischer, Ber., 1894, **27**, 3479.
3. Fischer, Z. Physiol. Chem., 1898, **26**, 60.
4. Haworth, Porter and Waine, J.C.S., 1932, 2254.
5. Fischer, Ber., 1895, **28**, 1145.
6. Bridel and Béguin, Compt. rend., 1926, **182**, 812.
7. Hérissey, Compt. rend., 1921, **172**, 766.
8. Fischer, Ber., 1901, **34**, 2896.
9. Fischer and Zach, Ber., 1912, **45**, 3761.
10. Moelwyn-Hughes, Trans. Faraday Soc., 1929, **25**, 81.
11. Willstätter and Oppenheimer, Z. Physiol. Chem., 1922, **121**, 191.
12. Willstätter, Kuhn and Sobotka, Z. Physiol. Chem., 1923, **129**, 33.
13. Helferich, Z. Physiol. Chem., 1932, **208**, 91.
    Helferich, Ergebnisse der Enzymforschung, II, 1933, 74.
14. Helferich, Rohr and Günther, Z. Physiol. Chem., 1933, **221**, 90.
15. Strauss, Dissertation, Halle, 1933.
16. Weidenhagen, Z. ver. Dtsch. Zuckerind, 1929, **79**, 591.
17. Petersen, Verhandl. Sächsischen Akad. Wiss., 1933. **3**, 154.
18. Helferich, Z. Physiol. Chem., 1933, **221**, 252.
19. Helferich and Becker, Ann., 1924, **440**, 1.
20. Helferich, Klein and Schäfer, Ber., 1926, **59**, 79.
21. Kuhn and Wagner-Jauregg, Z. Physiol. Chem., 19, **162**, 103.
22. Weidenhagen, Naturwiss., 1928, **16**, 654.
23. Weidenhagen, Z. ver. Deutsch. Zuckerind, 1930, **80**, 155.
24. Weidenhagen, Ergebnisse der Enzymforschung, 1932, I, 204.
25. Armstrong, Proc. Roy. Soc., 1904 **73B**, 516.
26. Michaelis and Menten, Biochem. Z., 1913, **49**, 333.
27. Grassmann, Stadler and Bender, Ann., 1933, **502**, 20.
28. Grassmann, Zechmeister, Toth and Stadler, Ann., 1933, **503**, 167.
29. Pringsheim and Ohlmeyer, Ber., 1932, **65**, 1243.
30. Armstrong, Proc. Roy. Soc., 1905, **76 B**, 600.
31. Lebedeff and Griaznow, Ber., 1912, **45**, 3256.
32. Bertrand, Ann. Chim. Phys. (8), 1904, **3**, 181.
33. Votoček, Valentin and Rac, Coll. Czech. Chem. Comm., 1930, **2**, 402.
34. Hermann and Neuschul, Biochem. Z., 1931, **233**, 129.
35. Péré, Ann. Inst. Pasteur, 1896, **10**, 417.
36. Raistrick and Rintoul, Phil. Trans., 1931, **220**, 1-367.
37. Butkewitsch, Jahr. Wiss. Bot., 1925, **64**, 637.
38. Bernhauer and Siebenäuger, Biochem. Z., 1930, **229**, 330.
39. Butkewitsch and Feodoroff, Biochem. Z., 1930, **229**, 87, 103.
40. Butkewitsch, Biochem. Z., 1929, **206**, 440 ; Biochem. Z., 1929, **207**, 302.
41. Chrzaszcz and Tiukow, Biochem. Z., 1930, **229**, 343.
42. Bernhauer, Oesterr. Chem. Z., 1931, **34**, 159.

43. Wehmer, Ber., 1925, **58**, 2616.
44. Wünschendorff and Kiliani, Compt. rend., 1928, **187**, 1572.
45. Challenger, J.C.S., 1927, **200**, 3044.
46. Angeletti and Cerruti, Ann. Chim. Applic., 1930, **20**, 424.
47. Butkewitsch, Biochem. Z., 1923, **142**, 195.
48. Challenger, J.C.S., 1929, 499; 1930, 16.
49. Quastel, Biochem. J., 1925, **19**, 652.
50. Kendall and Ishikawa, J. Infect. Dis., 1929, **44**, 282.
51. Koser and Saunders, Proc. Soc. Exp. Biol. Med., 1932, **30**, 218.
52. Avery and Heidelberger, G. Exp. Med., 1925, **42**, 367. Heidelberger and Goebel, J. Biol. Chem., 1927, **74**, 613.
53. Heidelberger and Kendall, J. Biol. Chem., 1932, **96**, 541.
54. Avery and Goebel, J. Exp. Med., 1933, **58**, 731.
55. Heidelberger, Goebel and Avery, J. Exp. Med., 1925, **42**, 701.
56. Butler and Cretcher, J.A.C.S., 1929, 1519.
57. Challinor, Haworth and Hirst, J.C.S., 1931, 258.
58. Dubos and Avery, J. Exp. Med., 1931, **54**, 51.
59. Dubos and Avery, J. Exp. Med., 1931, **54**, 73; 1932, **55**, 393; 1934, **59**, 641.
60. Maxim, Biochem. Z., 1930, **223**, 404.
61. Renfrew, J. Biol. Chem., 1930, **89**, 619.
62. Anderson, Z. Physiol. Chem., 1930, **191**, 172.
63. Goebel and Avery, J. Exp. Med., 1929, **50**, 521.
64. Tillett, Avery and Goebel, J. Exp. Med., 1929, **50**, 551.
65. Goebel and Avery, J. Exp. Med., 1931, **54**, 431.
66. Avery, Goebel and Babers, J. Exp. Med., 1932, **55**, 769.

# CHAPTER XIX.

## THE SYNTHESIS OF CARBOHYDRATES IN THE PLANT.*

THOUGH the primary facts of the photochemical assimilation of carbon dioxide by the green leaf are established, the full explanation of the process is still outstanding. Priestley (1771), Ingenhouse (1779) and Senebier (1788) established that green plants acquire their carbon from carbonic acid; De Saussure (1804), Boussingault (1861) showed that the volume of oxygen exhaled and that of carbon dioxide absorbed are approximately equal; Sachs in 1862 showed that the first visible product of the process is starch.

The very thorough researches of Willstätter and Stoll [1] showed that when the effect of respiration was eliminated, the "assimilatory quotient," that is the ratio of the volumes of oxygen released to carbon dioxide absorbed, is unity. The ratio is maintained under variations of temperature and carbon dioxide pressure much more extreme than are found under natural conditions.

The simple hypothesis advanced by Baeyer,[2] that formaldehyde is the first product of assimilation, later polymerising to carbohydrate, demands an assimilatory quotient of unity.

$$H_2O + CO_2 = HCHO + O_2 - 112{,}500 \text{ cals.}$$

Any other compound having the same composition could also be postulated, such as glycollic aldehyde, glyceric aldehyde or dihydroxy acetone, hexoses or even acetic acid.

The simplicity of the formaldehyde hypothesis has led to a very thorough search for it in the plant, but each time a positive result has been obtained, another experimenter has revealed the inadequacy of the technique of the first worker and obtained a negative result. If formaldehyde is supplied direct to green plants as a carbon source it acts as a poison, but according to Willstätter, it does not stop photosynthesis when present in low concentration.

Usher and Priestley,[3] Schryver,[4] and Klein and Werner [5] are among the authors who have obtained positive tests for formaldehyde.

* A full account of the early historical side of the question has been given by Meldola in a presidential address to the Chemical Society in 1906.

Negative results of equal weight have been obtained by Mameli and Pollaci,[6] Ewart,[7] Barton-Wright and Pratt,[8] and Vorländer.[9]

The best that can be said for the formaldehyde theory is that if it is produced as an intermediate, the rate of its transformation to carbohydrate is more rapid than its rate of formation, and it is too rapid to permit of its detection.

Similarly, there is equally little evidence for any other intermediate.

The function of chlorophyll is primarily as a photo-sensitiser. The reduction of carbon dioxide requires the supplying of a considerable amount of energy for it to proceed. The energy used is light energy, and since carbon dioxide and water do not absorb light in the visible region, chlorophyll intervenes to absorb the visible light and hand on the energy, in the same way in which certain dyestuffs are used to sensitise the silver bromide of the ordinary photographic plate to red light.

Many attempts have been made to obtain a photochemical reduction of carbon dioxide *in vitro*. Baly [10] in particular has claimed positive results, both for the formation of formaldehyde from carbon dioxide in ultra-violet light, and of organic compounds from the action of visible light on the finely divided carbonates of nickel and cobalt in carbonic acid solutions. The results seem difficult to reproduce and very specific instructions for the preparation of the catalyst must apparently be followed.[11]

Completely negative results on the lines of Baly's work have been obtained by many other workers, notably Zscheile,[12] Mackinney,[13] Emerson and Arnold,[14] Bell,[15] and Qureshi and Mohammed.[16]

One is inclined to agree with the summary of Spoehr [17] of the position of these experiments, that " it is difficult to avoid the conclusion that the burden of proof rests with those who maintain that positive results have been obtained."

In plants chlorophyll, which consists of two components, is localised in definite elements known as chloroplasts which contain in addition carotenoid pigments, proteins and lipoids. The condition of chlorophyll in the chloroplast is still a matter for dispute: it may be dissolved in the lipoids which are dispersed throughout the aqueous phase or it may be adsorbed on the surface of the proteins.

The intact cell is necessary for photosynthesis, which cannot be achieved with chlorophyll solutions nor even with the isolated chloroplasts.

It has never been established that chlorophyll intervenes chemically in photosynthesis, though it has usually been assumed that it does.

Bacteria which are carbon autotrophic adopt a variety of methods for reducing carbon dioxide and forming carbohydrates. Certain of them use the energy liberated in the oxidation of ammonia or nitrites for this purpose, while the green and purple sulphur bacteria obtain energy from the oxidation of hydrogen sulphide.

One of the most important and interesting problems in the plant is the nature of the first sugar to be formed. On the simplest theoretical grounds this would be expected to be glucose, but Brown and Morris,[18] in 1893, working with the leaves of *Tropæolum*, decided that sucrose was the first sugar to be synthesised by the assimilatory processes. It functions in the first place as a temporary reserve material, accumulating in the cell sap of the leaf parenchyma. As assimilation proceeds and the concentration of the cell sap exceeds a certain amount, which probably varies with the species of plant, starch is elaborated by the chloroplasts. This forms a more stable and permanent reserve material than the sucrose.

Parkin [19] selected the leaves of the snowdrop (*Galanthus nivalis*) for investigation, since this leaf does not form starch except in the guard cells of the stomata, though the bulb contains starch and inulin in abundance. Maltose was also proved to be absent from the leaf. He agreed with Brown and Morris.

A careful study of the carbohydrates of the mangold leaf and the potato leaf under actual normal conditions of growth was made by Davis, Daish and Sawyer [20] in 1916, whose papers summarise the work in this very difficult field. They confirmed the view of Brown and Morris that sucrose is the primary sugar formed.

Although this evidence was strongly in favour of the view that sucrose is the first sugar formed in photosynthesis, some observers held that hexoses are to be regarded as the primary products, sucrose being formed later by synthesis either in the leaf or in the root. Strakosch,[21] for example, employing microchemical methods, concluded that glucose was the first sugar formed in the mesophyll of the leaf. Weevers concluded the same from a study of variegated leaves.

Priestley [22] also was in favour of glucose.

Spoehr, in his book on photosynthesis, in 1926, regarded the evidence as still inconclusive and expressed the view that whilst glucose fitted the theoretical requirements most completely, yet the demonstration of its actually being the first sugar formed is still wanting.

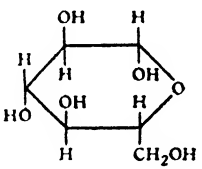
Barton-Wright and Pratt [23] criticise the experimental technique of Brown and of Davis, and show in particular that the methods employed for killing or suspending the activity of the plucked leaf were

faulty. It is best to chill this immediately to — 16°, and after cutting up to put it into water containing ammonia to extract the sugar. They determined the sugars hourly, using narcissus leaves. They found that the sugar is translocated to the flower and to the bulb as sucrose.

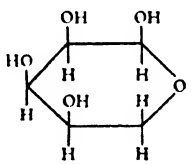
Their results afford evidence of a hexose being the primary sugar formed. Throughout the day there is a lag between increase in hexose and increase in sucrose. When, during rain, the rate of assimilation falls, so also does the production of hexose, but that of sucrose increases: there are three maxima in the daily curve for hexose, and after each there is a continuous rise in the quantity of sucrose. It is concluded, therefore, that glucose is the first product of photosynthesis, that somehow part of it becomes $\gamma$-fructose, and that the two appear united as sucrose.

Assuming that formaldehyde is the first product of the synthesis, two questions await an answer. Firstly, how is the condensation of the aldehyde caused; secondly, through what intermediate stages do the compounds pass?

Ordinary *d*-glucose, which appears to be the first sugar of photosynthesis, has the configuration in the $\beta$-form:—




$\beta$-*d*-glucose




Ribose

It is to be noted that the hydroxyl groups of adjacent carbons have a completely *trans* distribution, suggesting a regular assemblage of the $C_1$ or formaldehyde units from which the hexose is made up.

In the pentose sugar, ribose, the hydroxyl groups have all a *cis* configuration. Such a regular assemblage of formaldehyde groups is the extreme alternative to that found in the building up of $\beta$-glucose. Ribose, desoxyribose, and the corresponding alcohol, adonitol, are all natural products.

Theoretical considerations suggest that an isomer with a completely *trans* disposition of polar groups would be most stable. This would seem therefore to explain why glucose is the plant's first product and the commonest aldohexose. Unfortunately, accurate physical data, such as heats of combustion of the eight isomers, by which to test this hypothesis, do not exist.

The fact that the first step in plant synthesis is to produce the highly

asymmetric *d*-glucose raises the question why, and how, does the plant produce optically active compounds? Achievement of asymmetric syntheses *in vitro* makes it clear that once one asymmetric compound is introduced into a system, it directs the asymmetric synthesis of further compounds in that system.

Is the optical activity of glucose a necessary consequence of the photosynthetic mechanism, or is it determined by the asymmetry of chlorophyll itself? If the latter be true, optical activity is dictated by the hereditary pattern transmitted through the chromosomes, and has origin in the earlier stages of organic evolution.

Van't Hoff first suggested that circularly polarised light might play a part in the formation in nature of optically active compounds. Recently W. Kuhn [24] has succeeded with ultra-violet light of this kind in obtaining a partial asymmetric photochemical decomposition of racemates, and Mitchell [25] has achieved a similar decomposition using circularly polarised red light.

Mills [26] is sceptical of the polarised light theory; he considers that it is more profitable to inquire whether the property of growth which is characteristic of living matter may not necessarily lead to its dissymmetry. The chemical actions concerned in growth are completely stereospecific, and it is clear growth will proceed more rapidly in systems where each dissymmetric molecule encounters but one antimer of its dissymmetric co-reactant, than in a system where every dissymmetric component is present in both its antimeric forms.

If initially there were the slightest departure from exact equality of the *d*- and *l*-components of the system, this would increase cumulatively with growth until the one form practically disappeared. In seeking to explain how an original bias could have arisen, Mills reproduces a calculation which indicates that when 10 million dissymmetric molecules are produced under conditions which favour equally both enantiomorphs, there is an even chance that the product will contain an excess of more than 0·021 per cent. of one enantiomorph or the other. Indeed it is practically impossible for the product to be absolutely optically inactive. Ten million molecules of a catalyst of molecular weight 35,000, active in ·1 per cent. solution, would occupy a sphere of diameter 30$\mu$, which is ten times that of some of the smallest green and blue-green algæ.

It seems that one would have to postulate a convenient cataclysm for the selective destruction of those similarly constituted cells and their descendants which had developed the opposite series of enantiomorphs, in order to justify the Mills hypothesis. While the idea

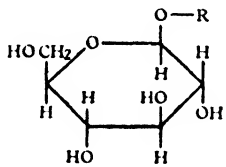
that the properties of living cells are in part due to their small size is a useful one, it cannot give an explanation of optical activity.

It has long been a subject for remark that of the possible stereoisomers of the simpler sugars, only a limited number are found in nature. Thus there occur

| | | | | |
|---|---|---|---|---|
| 4 | out of | 16 | possible | aldohexoses |
| 2 | ,, ,, | 8 | ,, | ketohexoses |
| 3 | ,, ,, | 8 | ,, | aldopentoses |
| 4 | ,, ,, | 16 | ,, | methyl pentoses |

In the following the hypothesis that the natural sugars arise by secondary transformation of the primary product *d*-glucose, and not by independent syntheses, will be discussed.

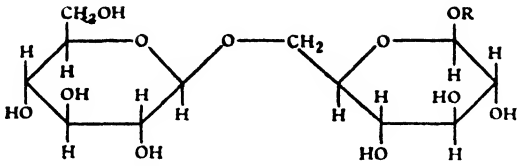
Glucose possesses in all its appearances the pyranose structure. It occurs free usually in association with fructose as invert sugar, and is combined with γ-fructose in sucrose and other oligosaccharides. It is found in many plants conjugated with hydroxyl or other groups of various substances as β-glucosides, which are synthesised by aid of the enzyme emulsin or by a more specific β-glucosidase peculiar to the plant.




The function of these compounds is diverse. In some the sugar acts as a solubilising group, enabling an undesirable substance to be transported and deposited in bark or root; in others it functions to transport a physiologically or biologically useful substance to the organ where it is desired, the liberation of the substance being controlled by a hydrolytic enzyme. Sometimes conjugation with sugar serves to protect easily oxidisable molecules, or possibly direct the condensation of intermediates in the synthesis of more complex molecules.

In discussing the " purpose " of a plant product, the distinction between functional and fortuitous occurrence is hard to draw and is usually artificial, and depends on one's particular biological philosophy or teleological beliefs.

In certain glycosides a second molecule of β-glucose is joined by its reducing group to $C_6$ of the first to form a gentiobioside :—



The most important polymer of glucose is cellulose, in which about 200 $\beta$-glucose units are joined in a regular chain.

The mechanism of formation of cellulose chains is unknown, no enzymic determinant having been discerned. If the building up occurred stepwise, it could proceed from either $C_1$ or $C_4$, but such an additive process seems improbable in that the reactive " end groups " would be exposed to destructive influences, and one would predict that cellulose chains of a great variety of length would be found. Molecular weight determinations by the ultra-centrifuge, however, show a high degree of homogeneity in chain length, which may of course be due to the method of preparation of the cellulose.

In the interpretation of the polymerisation of isoprene and of certain aldehydes under very high pressures, Conant [27] has suggested that the pressure orientates the molecules in a preferred manner and that these orientated molecules polymerise spontaneously by a chain mechanism. In the same way glucose molecules may be adsorbed and thus all orientated in a specific way on a surface in the cell (perhaps a cellulose micelle) and initiation of a chain reaction may cause a large number of molecules spontaneously to join, forming cellulose.

In the reserve polysaccharide starch, $\alpha$-glucose units form the building stone. Opinion is now almost reconciled to accept for starch a structure similar to that of cellulose, with the substitution of $\alpha$- for $\beta$-glucose units in the chain, which contains about 25 glucose units.

Fructose and glucose have an enolic form in common with the natural sugar mannose, which differs from glucose by the configuration of $C_2$. The three are easily interconvertible *in vitro* by the Lobry de Bruyn transformation, dilute alkali or even neutral phosphate acting on any one of them to give an equilibrium mixture, through formation of the enol and migration of a hydrogen.

Conditions similar to these might obtain in the plant, but it may be objected that mannose is not found in the free state as would be expected, nor in glycosides, but only in complex polysaccharides or mannosans whose distribution is limited to the hard skins and woody

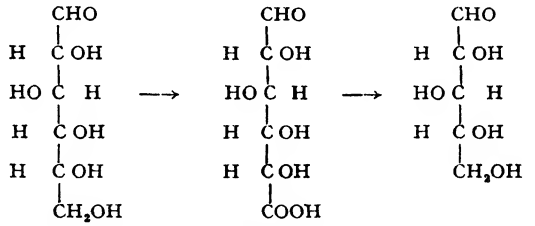
parts of seeds, sources which represent late products in biosynthesis. In these polysaccharides it is probable that anhydro β-mannose units are joined on the same pattern as the units in cellulose.

A possibility remains that the conversion from glucose to mannose occurs *in situ* in the polysaccharide chain.

The production of fructose from glucose has a possible explanation as an extrapolation from the results of Oparin and Kursanov discussed on page 198.

It may be suggested that fructose is not synthesised directly but it is obtained by hydrolysis of sucrose, which is produced by the condensation of glucose with the enolic hexosephosphate formed from glucose, and identical with that formed in fermentation from fructose and mannose.

The pentose xylose, though far less common than glucose, fulfils the same functions in its plant appearances, and is derived by replacement of the $CH_2OH$ group of the hexose by a hydrogen. There is strong circumstantial evidence that xylose owes its origin to the oxidation of the $CH_2OH$ group in a conjugated glucose to COOH (glucuronic acid), followed by decarboxylation.



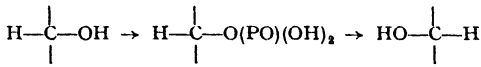

*d*-Glucose — *d*-Glucuronic acid. — *d*-Xylose.

*d*-Xylose rarely, if ever, is found free, but occurs in glycosides, especially in primeverosides, derivatives of 6-β-xylosidoglucose.

The polysaccharide xylan, which forms with cellulose the skeletal substance of wood and straw, has a structure exactly analogous to that of the cellulose, which it accompanies, together with polyglucuronates of similar structure. The hemicelluloses, which are components of incompletely formed woody substance, contain in association true cellulose chains, xylan and polyglucuronates. The properties of the last suggest they have a cellulose-like chain structure, and if this be true the biogenesis for xylose will be firmly established. The carboxyl groups of the polyglucuronates appear to be esterified and may so serve to knit together the long chains in the micelles. Chemical and X-ray work on cellulose has been carried out with purified samples

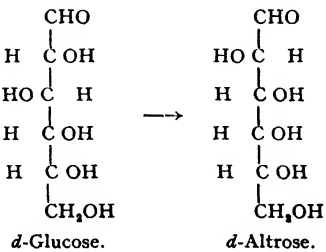
from special sources, such as cotton cellulose, but ordinary cellulosic material is a complex association and " celluloses " from different sources frequently contain COOH groups or analyse too low in water, pointing to inhomogeneity due to occasional $C_5H_8O_4$ units (anhydroxylose) in the chain.

The change from glucose to galactose involves the inversion of the groups attached to $C_4$. The mammary glands during lactation possess the power of doing this, and galactose is quite widely distributed in plants in which a similar mechanism must operate. Robinson [28] has suggested that it is possible to associate the change with the optical inversion which takes place on hydrolysis of a glucose-4-phosphoric acid.



He thus brings it into relation with the formation of hexose phosphates, which has been proved to be of such importance in alcoholic fermentation and other metabolic changes in plants and animals.

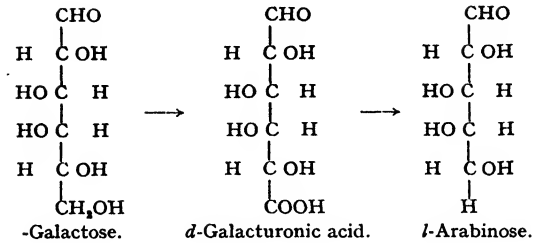
This theory lacks experimental proof, but it is of interest that a sugar transformation of this kind *in vitro* was discovered by Kunz and Hudson.[29] In the chlorination of lactose octacetate in the presence of active aluminium chloride a " neo-lactose " is produced, which has the structure 4-$\beta$-*d*-galactosido-*d*-altrose, the altrose arising by inversion at $C_2$ and $C_3$ in the glucose molecule.




Walden inversions are also proving to be of common occurrence during the hydrolysis of toluene sulphonyl derivatives of the sugars.

*l*-Galactose has been discovered for the first time in flax seed mucilage.

The pentose *l*-arabinose is related to *d*-galactose exactly as *d*-xylose is to *d*-glucose, and so a similar biogenesis by decarboxylation is probable, and well in agreement with the facts. The $CH_2OH$ group of galactose is much more readily oxidised than that of glucose.



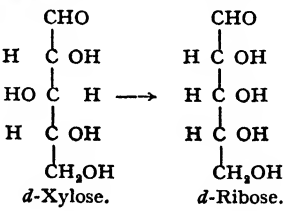

Arabinose has a source in glycosides, particularly in vicianosides (6-$\beta$-*l*-arabinosido-glucose), analogous to gentiobiosides and primeverosides. Its main source is in the exudations which are plant gums and in the pectins, in which it occurs as a polymer in a complex association.

Pectins constitute up to one-half of the dry weight of fleshy fruits, leaves and roots, but are found only in traces in woody material. They play an important part in the water balance of the plant, and consist of calcium and magnesium salts of a complicated carbohydrate association in which the intimate association of *d*-galactose, *d*-galacturonic acid and *l*-arabinose is strong evidence for the suggested biosynthesis of these substances. In a growing plant pectin content bears an inverse relation to lignin, the latter possibly arising from the former.

There are two isolated occurrences of *d*-arabinose in nature, the sugar which in the laboratory results on elimination of $C_1$ of *d*-glucose by the Wohl or Ruff degradative methods. These are in the glycoside barbaloin and in the polysaccharide of tubercle bacillus. In these rare instances the pentose may originate from *d*-glucose by elimination of $C_1$.

The natural pentose *d*-ribose is not configurationally related to any of the natural hexoses. Its distribution is very specialised, being restricted to the nucleosides of plants and animals, 2-desoxy ribose being the commoner sugar in animal nucleosides.

A special distribution suggested to Robinson [28] a special origin. It was suggested by him that *d*-ribose did not exist as such in nucleotides, but was formed by Walden inversion on hydrolysis of the phosphoric acid group on $C_3$ of the common natural pentose *d*-xylose.



Levene[80] did not accept this explanation, and has since shown that in adenosine nucleotide the sugar has a $\gamma$-ring because the phosphoric acid group is attached to $C_5$, and not to $C_3$ as the Robinson theory requires.

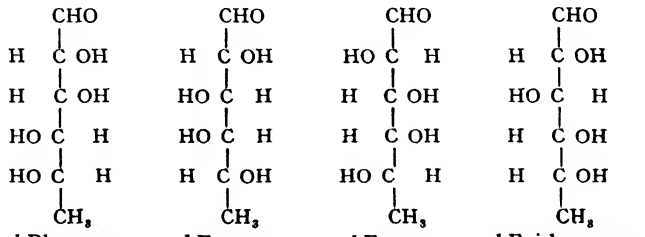
Ribose therefore exists as such in nucleotides, but of course may arise from *d*-xylose at a previous stage. A separate synthesis is by no means excluded, and indeed likely in view of what has been said earlier of the unique stereochemical configuration of ribose, and of the unique occurrence of ribose in the cell nuclei.

Three hexitols found in nature are the alcohols derived by the reduction of glucose, mannose and galactose.

Sorbitol from glucose is chiefly found in *Rosaceæ*, in apples, pears, plums, cherries and in mountain ash: the 1 : 5 anhydride form styracitol is also known, suggesting that it has been formed by the direct reduction of glucopyranose.

Mannitol is the commonest hexitol, occurring widely in all families of plants derived perhaps by the asymmetric reduction of fructose rather than by reduction of mannose. The first step in the utilisation of glucose by many species of *Aspergillus* is the disproportionation of two molecules to give mannitol and gluconic acid respectively, an instance of the specificity of a biological transformation.

The methylpentoses may in theory be derived from the hexoses by substitution of $CH_3$ for the $CH_2OH$ group. Four are known from plant sources. Three of these are found only in glycosides, *d*-fucose and *d*-epirhamnose being of very rare occurrence, while *l*-rhamnose has a wide distribution. *l*-Fucose appears in the cell wall material of seaweeds in the polymer fucosan, about which very little is known.




*Systematic name :—*

| *l*-Manno-methylose. | *d*-Galacto-methylose. | *l*-Galacto-methylose. | *d*-Gluco-methylose. |
|---|---|---|---|

The very rare *d*-fucose is related to *d*-galactose, and the rare *d*-epirhamnose to *d*-glucose, but the commonly found *l*-rhamnose is related to *l*-mannose, and *l*-fucose to *l*-galactose, neither of which are found in nature.

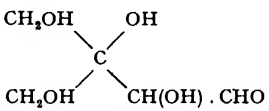
A simple reduction of the appropriate aldohexose is therefore inadequate to explain the biosynthesis of all the methylpentoses. Freudenberg [31] points out that by arbitrary assumptions one can derive the methylpentoses by oxidation of hexitols or disproportionation of the cyclitols, but concludes that there is no satisfactory explanation for the origin of *l*-rhamnose; this indeed is an unsolved problem. Rhamnose, where it is associated with other sugars, is found with galactose.

It is now relevant to sum up the evidence presented in detail, in which all simple sugars of ascertained structure have been examined from the view point that since in general they were configurationally related to *d*-glucose, they were derived from it in plants and not formed independently.

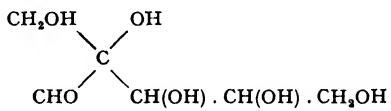
For those carbohydrates of wide distribution it is maintained that the evidence strongly supports this hypothesis. Certain sugars are structurally unrelated to *d*-glucose and cannot be derived from it, and must arise in other ways. All these sugars have a limited distribution or occur in a specialised biological product, and so for them a separate synthesis is admissible. (Ribose, digitoxose, etc.)

An unexplained anomaly is the biogenesis of *l*-rhamnose.

Units for the synthesis of the rarer sugars are provided by the breakdown of hexose to biose and triose, as in fermentation; proof of their occasional intervention is given by the branched chain sugars, apiose, from the glycoside apiin from parsley, and hamamelose from hamameli tannin:



Apiose.



Hamamelose.

and the curious "disaccharide" found by Buston and Schryver in cabbage leaves:

$$CH_2OH\ CH(OH) - O - CH_2OH.$$

## REFERENCES TO CHAPTER XIX.


1. WILLSTÄTTER AND STOLL, *Untersuchungen über die Assimilation der Kohlensäure*, Berlin, 1918.
2. BAEYER, Ber., 1870, **3**, 63.
3. USHER AND PRIESTLEY, Proc. Roy. Soc., 1906, **77 B**, 369; Proc. Roy. Soc., 1911, **84 B**, 101.
4. SCHRYVER, Proc. Roy. Soc., 1910, **82 B**, 226.
5. KLEIN AND WERNER, Biochem. Z., 1926, **168**, 361.
6. MAMELI AND POLLACI, Atti R. Accad. Lincei, 1908, **17**, 739.
7. EWART, Proc. Roy. Soc., 1908, **80 B**, 30.
8. BARTON-WRIGHT AND PRATT, Biochem. J., 1930, **24**, 1210.
9. VORLÄNDER, Planta, 1928, **6**, 684; Z. Anal. Chem., 1929, **77**, 241.
10. BALY, Ind. Eng. Chem., 1924, **16**, 1016.
11. BALY, Trans. Faraday Soc., 1931, **27**, 545.
12. ZSCHEILE, J.A.C.S., 1932, 973.
13. MACKINNEY, J.A.C.S., 1932, 1688.
14. EMERSON AND ARNOLD, J. Gen. Physiol., 1932, **15**, 391.
15. BELL, Trans. Faraday Soc., 1931, **27**, 771.
16. QURESHI AND MOHAMMED, J. Phys. Chem., 1932, **36**, 2205.
17. SPOEHR, Annual Review of Biochemistry, 1933, **2**, 466.
18. BROWN AND MORRIS, J.C.S., 1893, 604.
19. PARKIN, Biochem. J., 1911, **6**, 1.
20. DAVIS, DAISH AND SAWYER, J. Agric. Science, 1916, **7**, 255; 327; 352.
21. STRAKOSCH, Sitzungsber. K. Akad. Wiss. Wien, 1907, **116**, 855.
22. PRIESTLEY, New Phytologist, 1924, **23**, 255.
23. BARTON-WRIGHT AND PRATT, Biochem. J., 1930, **24**, 1210.
24. KUHN, Naturwiss., 1929, **17**, 227; Naturwiss., 1930, **18**, 183.
25. MITCHELL, J.C.S., 1930, 1829.
26. MILLS, Chem. and Ind., 1932, p. 756.
27. CONANT, J.A.C.S., 1932, 628.
28. ROBINSON, Nature, 1927, **120**, 44; 656.
29. KUNZ AND HUDSON, J.A.C.S., 1926, 1978; 2435.
30. LEVENE, Nature, 1927, **120**, 621. LEVENE AND TIPSON, J.B.C., 1932, **94**, 809.
31. FREUDENBERG AND RASCHIG, Ber., 1929, **62**, 373.

# INDEX.

*NOTE.—The references in heavy type denote the more detailed descriptions of the compounds or subjects indexed.*

PRINTED IN GREAT BRITAIN BY THE UNIVERSITY PRESS, ABERDEEN